National Institute of Neurological Disorders and Stroke

INTRAMURAL RESEARCH ANNUAL REPORT 1994

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02875-02 NS

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Molecular Genetics of Human Dementias

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Lev G. Goldfarb, M.D., Ph.D.	Visiting Scientist	NS, BNP, DIR, NINDS
Others:	Olavo Vasconcelos, M.D.	Visiting Fellow	NS, BNP, DIR, NINDS
	Mark Dubnick, Ph.D.	Senior Staff Fellow	NS, BNP, DIR, NINDS
	James Nagle	Biologist	NS, BNP, DIR, NINDS
	Juraj Cervenak, M.D.	Special Volunteer	NS, BNP, DIR, NINDS

COOPERATING UNITS (*if any*)
Paul Brown, M.D., Larisa Gervenakova, Ph.D., D. Carleton Gajdusek, M.D., LCNSS, NINDS

LAB/BRANCH
Office of the Director, BNP, DIR

SECTION
, Neurogenetics Section, BNP, DIR,

INSTITUTE AND LOCATION
National Institute of Neurological Disorders and Stroke, Park Building

TOTAL STAFF YEARS: 2.2	PROFESSIONAL: 1.7	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[x] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Seventeen different germline mutations in the PRNP gene on chromosome 20p have been identified in patients with familial Creutzfeldt-Jakob disease (CJD). Of these, an independent mutation (a 48-bp insertion) was discovered and a corresponding phenotype described, and two other complex pathogenic alleles causing fatal familial insomnia were characterized within the last 12 months. A polymorphism at codon 129 was shown to control the age of onset and severity of illness in familial CJD cases and to regulate susceptibility in sporadic and iatrogenic CJD. The codon 129 polymorphism in the PRNP gene does not influence phenotypic expression of other neurologic disorders. Similarly, a polymorphism in the apolipoprotein E gene on chromosome 19 that has been shown to genetically control the age of onset in sporadic Alzheimer's disease, does not influence the age of onset or the rate of progression in CJD. Synthetic peptides that included mutant amino acids at positions 178 and 200 form abundant amyloid fibrils with a tendency to aggregate into large amyloid complexes. In addition, the mutant peptides accelerate fibrillogenesis by the peptides with wild type sequences. These findings are consistent with the hypothesis that specific mutations may alter the folding of the normal host protein to favor formation of insoluble and protease resistant amyloid fibrils.

PHS 6040 (Rev 1 84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02876-02 NS

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Movement Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Lev G. Goldfarb, M.D., Ph.D.	Visiting Scientist	NS,BNP, DIR, NINDS
Others:	Olavo Vasconcelos, M.D.	Visiting Fellow	NS, BNP, DIR, NINDS
	Mark Dubnick, Ph.D.	Senior Staff Fellow	NS, BNP, DIR, NINDS
	Tinatin Chabrashvili, M.D.	Visiting Fellow	NS, BNP, DIR, NINDS
	James W. Nagle	Biologist	NS, BNP, DIR, NINDS

COOPERATING UNITS *(if any)*

J.J. Higgins, M.D., M. Hallett, M.D., C. Toro, M.D., L. Nee, M.S.W., CNB; D.C. Gajdusek, L. Cervenakova, LNCSS, V.P. Alexeev, M.D., F.A. Platonov, M.D., S. Kononova, Ph.D., VE Center, Yakutsk; A. Lunkes, University of Dusseldorf.

LAB/BRANCH

Office of the Director, BNP, DIR

SECTION

Neurogenectics Section, BNP, DIR,

INSTITUTE AND LOCATION

NINDS, Park Building

TOTAL STAFF YEARS: 2.2	PROFESSIONAL: 1.7	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the Siberian kindred of segregating autosomal dominant cerebellar ataxia, 131 patients were characterized clinically and 2 pathologically. The collected pedigrees include 1235 individuals, of which 360 are at a 50% risk and 242 at a 25% risk of having the disease. Ataxia in this kindred has been genetically linked to the SCA1 gene on chromosome 6p and the pedigree screened for the recently described CAG repeat expansion in this gene using GeneScan program (ABI). The normal allele had 20to 37 repeat units and a CAT or CATCAGCAT interruption between the first and the second sequences of 10 to 17 CAG repeats. The pathogenic allele was extended to 39 to 60 uninterrupted repeats in all 59 analyzed ataxia patients. The number of repeat units correlated inversely with the age of disease onset. Repeat numbers of 43 to 55 were also found in 39 of 105 tested unaffected first and second degree relatives. Homozygosity for the elongated allele was observed in two affected individuals and an unaffected child; the age of onset and clinical course were no different from the heterozygotes carrying alleles with a similar number of repeat units.

Testing for the SCA1 gene mutation has been performed in 44 American families with ataxia; only two were shown to have a CAG triplet expansion. Genetic typing of a large family from Virginia indicated chromosome 14q as a probable gene location. Further attempts to identify mutations in American ataxia families are underway. Another large Virginian family tracing its ancestry to Colonial America and a family from Texas expressing essential tremor and focal dystonia are under genetic linkage study with the use of highly polymorphic minisatellite markers. The size and uniform clinical expression make these families uniquely suitable for genetic identification.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02906-01 NS

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dissecting the Genetics of Complex Neurologic Traits

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	L.D. Hudson, Ph.D.	Chairperson, Basic Neurogenetics Program	NS, BNP, DIR, NINDS
Others:	M.A. McKee	Commissioned Officer	NS, BNP, DIR, NINDS
	N. Tayebi	IRTA	NS, BNP, DIR, NINDS
	G. Szabo	Research Volunteer	NS, BNP, DIR, NINDS

COOPERATING UNITS *(if any)*
H. Arnheiter, Viral Pathogenesis Section, LVMP, NINDS; J. Barker, Lab of Neurophysiology, NINDS; W. Theodore, Clinical Epilepsy Branch, NINDS; H. McFarland, Neuroimmunology Branch, NINDS

LAB/BRANCH
Basic Neurosciences Program

SECTION
Section of Neurogenetics

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The genetic dissection of a complex trait involves identifying a group of genes that contribute to the expression of a given phenotype. Our group has initiated a genetic analysis of two common neurologic traits, epilepsy and multiple sclerosis (MS). Defining a collection of genes, each of which is able to influence a complex trait such as epilepsy, will enable the construction of a wall chart of genetic interactions that may provide insights as to the mechanisms underlying normal communication between neurons. Our strategy includes both a candidate gene and a positional cloning approach, and the experimental systems include mouse and man. In order to identify genes that result in or predispose to epilepsy, one of the candidate genes will first be knocked out in transgenic mice. The gene encoding the GABA synthetic enzyme, glutamic acid decarboxylase (GAD), is being targetted for elimination because GABA-ergic inhibitory mechanisms normally restrict development of firing bursts typical of epileptic seizures. Embryonic stem cells are currently being screened for homologous recombination at the GAD locus. Any transgenic mice that display seizures will be employed to isolate "modifier" genes that influence this phenotype. This will be carried out by crossing the GAD knock-out onto a number of inbred mouse strains and performing linkage analysis to close in on other genes that affect the epileptic phenotype. The cognate human genes for these loci that contribute to an epileptic phenotype can then be cloned. In a second approach to defining epilepsy genes in man, families with an inherited, defined pattern of seizures are undergoing clinical evaluation in preparation for positional cloning. The focus is on families with Rolandic epilepsy, a type which is inherited as an autosomal dominant with a characteristic wave form of seizures.

A candidate gene approach is also underway for MS. The inability to synthesize or maintain the myelin sheath in this demyelinating disorder could arise from defects in any of a number of oligodendrocyte proteins or in cytokines thought to be critical for myelination. The existence of a number of families with a high incidence of MS has allowed us to screen for linkage of the disease trait to the myelin basic protein (MBP) gene, the proteolipid protein (PLP) gene and other candidate genes.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02890-02 SMS

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Calcium Channels in Vertebrate Nerve Terminals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	E.F. Stanley, Ph.D.	Staff Physiologist, SMS
Others:	Yuan Liu, Ph.D.	Senior Staff Fellow, SMS
	Paul Joseph Church, Ph.D.	IRTA Fellow, SMS
	Wolfram A. Gottschalk	Pre-IRTA Fellow, SMS

COOPERATING UNITS *(if any)*

P. Haydon, Ph.D. Department of Zoological and Molecular Biology, Univ. Iowa, Ames, IA, H. Chin, Ph.D., Staff Scientist, LNC, NINDS

LAB/BRANCH

SECTION

Synaptic Mechanisms Section, BNP, DIR

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892.

TOTAL STAFF YEARS: 4.8	PROFESSIONAL: 4.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Information is transferred from one neuron to the next at synapses, points of intimate contact, by the release of a chemical neurotransmitter. While it is well established that the entry of calcium through ion channels is a critical step in the release of the transmitter, the mechanism and the modulation of this process remains poorly understood. We have previously demonstrated that the calyx type presynaptic terminal of the chick ciliary ganglion can be used to record single calcium channel activity at a presynaptic nerve terminal release face. We have used this preparation to show that single quanta of transmitter can be released during single calcium channel activity. This result is strong evidence that the calcium channel and the transmitter release mechanism are very closely situated, presumably as part of a multimolecular complex. The calyx was also used to demonstrate a regular spacing of individual calcium channels in the transmitter release face using atomic force microscopy. We have also made the first direct recordings of ligand-gated ion channels, activated by ATP, from a presynaptic nerve terminal. These channels may be important in the feedback modulation of transmitter release.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02895-01 OCD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Neuromuscular Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark Hallett, M.D.	Clinical Director		OCD	DIR	NINDS
Others:	Carlos Luciano, M.D.	Act. Chief, EMG Lab	EMG	OCD	DIR	NINDS
	Carlos Otero, M.D.	Clinical Associate	EMG	OCD	DIR	NINDS
	Mary Kay Floeter, M.D.	Senior Staff Fellow	EMG	OCD	DIR	NINDS
	James Russell, M.D.	Visiting Associate	ENG	OCD	DIR	NINDS
	Nguyet Dang	Biomedical Engineer	EMG	OCD	DIR	NINDS
	Marinos Dalakas, M.D.	Chief	NDS	MNB	DIR	NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Clinical Director, CNP, DIR

SECTION

Electromyography (EMG) Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.3	1.7	0.6

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This protocol was developed as an effort to characterize new neuromuscular diseases, to learn more about established diseases, to assess current methodologies and technologies and to refine old methods as well as to develop new ones. Using different electrodiagnostic techniques, we have carried a number of different studies:

a. Correlation between muscle histology and quantitative electromyography in zidovudine-induced myopathy. We have found that most of the electromyographic abnormalities are not specific for myopathy but correlate with some zidovudine-induced histologic changes (ragged red fibers) and inflammation.

b. Electrophysiologic studies in inclusion body myositis to assess for the possibility of a coexistent neurogenic component. Macro-electromyography (macro-EMG) a recently developed technique, that provides information about the territory of the motor unit, has not shown, on a preliminary assessment, evidence of significant reinnervation.

c. Reinnervation in clinically unaffected muscles of patients with prior paralytic poliomyelitis: correlation between macro-EMG and histology. We have found a positive correlation between the amplitude of the motor unit potential recorded with the macro-EMG technique and histologic evidence of reinnervation such as fiber-type grouping.

d. Electrophysiologic and quantitative sensory studies in patients with Fabry's disease. We have found evidence of predominant involvement of small myelinated axons with sparing of large myelinated axons as assessed with quantitative sensory studies and nerve conduction studies.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01424-28 OCD

PERIOD COVERED

October I, 1993 through September 30,1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral Modulation by the Limbic System in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
Others:	A. August, M.A.	Psychologist	CNU,OCD, NINDS
	C. Kufta, M.D.	Medical Officer	SNB, NINDS
	S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS

COOPERATING UNITS *(if any)*

Surgical Neurology Branch, DIR, NINDS
Office of the Clinical Director, NINDS

LAB/BRANCH

Office of the Clinical Director

SECTION

Clinical Neuropsychology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
- [ ] (a1) Minors
- [ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interictal behavior of patients with temporal lobe epilepsy (TLE) have been charted by various psychosocial and neuropsychiatric scales. To date, the evidence aligns left (LTE) and right (RTE) temporal epilepsy along axes of dysphoria-euphoria, catastrophic-denial reactions and hypo-hyperarousal, respectively. The profile of patients with LTE may be characterized as socially distant and negative, overly anxious, and colored by diminished self-esteem and lower sense of personal behavior; the converse may be true for RTE patients who advance a more positive image.

In spite of these robust efforts to quantify interictal emotional or behavioral traits of TLE patients, relatively little attention has been dedicated to systematically evaluate the psychological effects and behavioral changes following interventive treatment such as temporal lobectomy. The present study draws comparisons of patients before and after unilateral lobectomy, and focuses on their enduring personality traits (Axis II) and self-perception in relation to internal and external frames of reference.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01245-29 OCD

PERIOD COVERED
October 1,1993 through September 30,1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
EEG Learning Correlates Using Scalp and Intracranial Electrodes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD NINDS
Others:	S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS
	A. August, M.A.	Psychologist	CNU, OCD, NINDS
	C. Kufta, M.D.	Medical Officer	SNB, NINDS

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, DIR, NINDS

LAB/BRANCH
Office of the Clinical Director

SECTION
Clinical Neuropsychology Unit

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.71	PROFESSIONAL: 0	OTHER: 0.71

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electrophysiologic responsivity (skin conductance and EEG) were monitored from patients with temporal lobe epilepsy during challenging procedures. There appeared to be greater frontal EEG activation, right more than left, during emotionally evocative stimulation and feedback about failing or passing and experimental test. Failure was more evocative to left, and passing was more evocative to right, brain injured patients.

EEG recordings taken after intracarotid amytal injection showed frontal changes in patients who displayed emotional reactions. These data suggest that the euphoria and dysphoria following brain injury may reflect ipsilateral dysinhibition. This model posits that positive and negative emotions may be better modulated by left and right brain mechanisms, respectively.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00200-40 OCD

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Cognitive and Emotional Profile of Neuropsychiatric Disorder

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
Others:	A. August, M.A.	Psychologist	CNU, OCD, NINDS

COOPERATING UNITS (*if any*)

LAB/BRANCH

Office of the Clinical Director

SECTION

Clinical Neuropsychology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.94	PROFESSIONAL: 0.50	OTHER: 0.44

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Experiments using brain stimulation, PET imaging and behavioral procedures were initiated to identify the neuroanatomic basis of memory and language disorders.

Functional mapping of cortical and subcortical sites in patients with epilepsy (23) or Parkinsonism (12) established Wernicke's area as the primary mediator of language, working memory, and related experimental memories. With pulvinar stimulation, naming and working memory were less compromised and patients often remembered their difficulty. While basotemporal stimulation evoked anomia, patients accurately recalled both the experience and specific item they had missed, suggesting a greater role in language than in memory. Overall, if patients failed to name items during stimulation, they were less likely to recall the experiences; however, if they misnamed, their memory for the events and items improved, indicating that initial activation of semantic systems is an important factor in working and experiential memory.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02151-20 LAS

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Memory Storage in Neural Networks

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D.L. Alkon, M.D. Medical Officer BNP, DIR, NINDS

Others: T. Nelson, Chemist; D Dahl, Biologist; R. Etcheberrigaray, Vis. Sci; Carlos Collin, Vis. Sci.; L Wang, Vis. Assoc.; B. Schreurs, Sr. Staff Fellow; J. Olds, Sr. Staff Fellow; J Schachter, Staff Fellow; O Zohar, Vis. Fellow; N Hirashima, Vis Fellow; A Favit, Vis Fellow; N Meiri, Vis Fellow; C Yi, Vis Fellow; C.J. Lee, Vis. Fellow; Y-F Han, Vis. Fellow; D. McPhie, IRTA Fellow; K.L. Blackwell, Guest Res.; M. Boakye, Guest Res.; D Lester, Guest Res; C Kim, Spec. Vol; C Hirashima, Spec. Vol; M Oh, Spec. Vol.; A Hutter, Spec. Vol.; V Kowtha, Spec. Vol; K. Kusuzaki, Spec Vol.

COOPERATING UNITS *(if any)*

Marine Biological Laboratory, Woods Hole, MA 02543 (A. Kuzirian); California Institute of Technology (C. Chen); Medical Research Council, Canada (B. Bank)

LAB/BRANCH

Laboratory of Adaptive Systems

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 18.53	PROFESSIONAL: 18.03	OTHER: 0.50

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our principal objective is to define molecular and biophysical mechanisms of associative learning and memory. Emphasis is placed on learning and memory which can be related to human cognition. Ultimate goals are to arrive at clinically meaningful interventions and to design and construct artificial intelligence which has advanced learning and memory capabilities. With human cognitive function as the principal frame of reference, the research focuses on associative processes (such as Pavlovian conditioning) rather than non-associative behavioral modifications (such as sensory adaptation, habituation, arousal, and sensitization). The biological basis of learning and memory is of interest at several levels of complexity: behavior, neuronal systems, neuronal architecture and membranes and molecular transformations. To reconstruct the physiology involved (and to model it in artificial contexts) it is necessary to use both "simple system" preparations such as the nudibranch mollusk *Hermissenda crassicornis* as well as "complex system" preparations such as rabbits and rats. The molluscan work has yielded the first unequivocal biological record of an associative memory. This record consists of persistent transformations of specific ionic channels. Because these records have been found within the membranes of identified single neurons, it is now possible to define biochemical pathways regulating such long-term membrane modifications as well as to analyze how this biological memory record is expressed by the integrative functions of an entire neuronal system. The work on the vertebrate brain offers two essential opportunities. First, the generality of mechanisms determined for much less evolved species can be tested. Remarkably, the same ionic channel transformations were shown to record associative memory in the rabbit as were found in *Hermissenda*. Rabbit and now rat neural systems have also provided sufficient quantities of tissue so that conditioning-specific alterations of critical enzymatic (e.g., protein kinase C) pathways which control membrane excitability have recently been demonstrated. Furthermore, identical G protein substrates which regulate similar K^+ channels, intraaxonal transport, mRNA turnover, and architecture of dendritic trees, undergo memory-specific modification in mollusks and mammals. Such biophysical and molecular parallels in mechanisms of memory storage suggest the possibility of general cellular principles of memory storage significant for human physiology and pathophysiology as well. These identified conserved mechanisms of associative memory are guiding a program to uncover targets of dysfunction in Alzheimer's disease for purposes of diagnosis, therapy, and prevention.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01282-30 LCNSS

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of Population Isolates: Study of Child Growth, Development, Behavior and Learning, and Disease Patterns in Isolated and Primitive Groups

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Carleton Gajdusek, M.D.	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Supv. Research Biologist	LCNSS
	Richard Yanagihara, M.D.	Medical Director	LCNSS

COOPERATING UNITS (if any)
Continued

LAB/BRANCH
Laboratory of Central Nervous System Studies

SECTION

INSTITUTE AND LOCATION
NINDS, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 13.2	PROFESSIONAL: 8.1	OTHER: 5.1

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[x] (a1) Minors
[x] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of human biology of vanishing primitive societies focus on neurologic development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which most of our studies evolved: kuru-CJD GSS-FFI, HIV (AIDS), HTLV-I slow virus infections of the CNS, aging and Alzheimer's, VE, ALS/PD, mental disease, toxic neuropathies. Techniques of molecular genetics, biochemistry, immunology, virology, and field epidemiologic, clinical linguistic and behavioral studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens from expeditions to Micronesia, Melanesia, Polynesia, South America, Asia and Africa were valuable in recent HIV (AIDS), HTLV-I hantavirus, JCV of PML and herpesvirus, CMV and EBV studies. Studies on nutrition, reproduction, fertility, age of puberty and aging, genetic distance and pleomorphisms, unusual and odd higher cortical functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we cannot investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PD, HTLV-I myelopathy, epilepsy, familial parkinsonism, Viliuisk encephalopathy, other CNS degenerations, hysterical disorders, schizophrenia, bipolar psychoses, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections in these isolated groups have yielded widely significant discoveries. HFRS caused by hantaviruses in Asia, USSR, Europe and newly recognized hantaviruses in the U.S. are studied. Human evolution and adaptability to high altitude, various climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social psychologic stress are studied in appropriate populations. Thus, HTLV-1 and HIV retroviruses as causes of CNS diseases in man were first found and are best studied in isolated or socially segregated groups: high incidence TSP focus in Tuamaco, Colombia; drug-using mothers in Newark, New Jersey; epidemic neuropathy in Cuba. We new have a proto-Melanesian variant of HTLV-I in New Guinea and Solomon Islands, of an archaic origin, not associated with monkeys at least for millenia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 00969 30 CNSS

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Carleton Gajdusek, M.D.	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Supv. Research Biologist	LCNSS
	Richard Yanagihara, M.D	Medical Director	LCNSS

COOPERATING UNITS *(if any)*
Continued

LAB/BRANCH
Laboratory of Central Nervous System Studies

SECTION

INSTITUTE AND LOCATION
NINDS, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 19.3	PROFESSIONAL: 10.8	OTHER: 8.5

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[x] (a1) Minors
[x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies focus on causes and pathogenesis of chronic degenerative CNS disorders with emphasis on MS; Parkinson's, Pick's, Huntington's and Alzheimer's diseases; ALS; ALS/PD of Western Pacific; supranuclear palsy; other presenile dementias; spinocerebellar ataxias; epilepsy; chronic encephalitis with focal epilepsy; Viliuisk encephalopathy; muscular dystrophies; chronic schizophrenia; bipolar psychoses, autism; SSPE; PML; dialysis encephalopathy, goiterous cretinism; cysticercosis; and intracranial neoplasms. We have defined the transmissible and nontransmissible dementias as brain amyloidoses caused by post-translational modification of a specific host precursor protein to amyloid fibril deposits. We now recognize the slow unconventional viruses causing kuru-CJD-scrapie as replicating polypeptides formed *de novo* from a normal host precursor protein, specified on chromosome 20 in man and 2 in mice. The molecular elucidation of the spontaneous conformational change to infectivity, basically a crystallographic problem, is now becoming our major target. Molecular genetic analysis of familial CJD already indicates several point mutations which enormously increase (x10) the probability of this spontaneous *de novo* conversion to an infectious polypeptide. Microbiology must now contend with a totally new paradigm for replicating, infectious, pathogenic agents in the transmissible brain amyloidoses. Our studies focus on the elucidation of the molecular configurational events conferring the property of infectivity on a previously normal host precursor using CD spectrophotometry, high-voltage EM, MRI to elucidate the change in conformation which occurs as transmissibility is produced. In normal aging, Alzheimer's disease (AD), and Down's syndrome, a different host precursor protein (specified on chromosome 21 in man, 16 in mice) is a cell-excreted inhibitor of growth factors (protease nexin II). Posttranslational degradation of this normal precursor forms the 42-amino acid amyloid polypeptide which polymerizes to form the deposits of amyloid angiopathy, amyloid plaques and neurofibrillary tangles in aging, AD and Down's. This occurs in all individuals who reach their 90s. Genetic, toxic, and infectious factors may accelerate this aging brain amyloid deposition.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02549-13 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Herpesvirus Infections and Nervous System Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS
Others:	S. Keir, Ph.D.	Visiting Fellow	LENP, NINDS
	W.J. Mitchell, D.V.M., Ph.D.	Sr. Staff Fellow	LENP, NINDS
	D.B. Henken, Ph.D.	Sr. Staff Fellow	LENP, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS *(if any)*

Anatomic Pathology, Texas Childrens Hosp. (C. Langston, M.D.); Dept. of Pediatrics, Univ. of Alabama at Birmingham (E. Kern, Ph.D.)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
2.37	1.97	0.40

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project, nervous system disease associated with human herpesvirus infections are examined. Agents include neurotropic herpes simplex virus types 1 and 2 (HSV-1,-2) and varicella zoster virus (VZV), as well as other human herpesviruses known or suspected to infect the nervous system (cytomegalovirus [CMV], Epstein-Barr virus [EBV], and human herpesvirus types 6 and 7 [HHV-6,-7]). For neurotropic herpesviruses, experimental models are used to examine mechanisms underlying production of neural lesions. Problems of particular interest include: the role of infection with HSV, VZV and other herpes viruses in the production of CNS and PNS disease, including (a) acute encephalitis, (b) infection during nervous development, (c) chronic demyelination, and (d) mechanisms of CNS arteritis and stroke induced by neurotropic herpesviruses.

During FY 1994, experiments to localize herpesvirus DNA sequences in tissue sections from experimentally infected animals and human autopsy tissues using an *in situ* polymerase chain reaction (ISPCR) method were continued.

A study to identify the cell type containing HSV DNA in the mouse trigeminal root entry zone during acute infection and post-acute period was completed. By ISPCR, HSV DNA sequence-containing nuclei were associated with GFAP-positive process by immunohistochemistry. This colocalization study shows that astrocytes can harbor HSV DNA long-term, and is evidence for persistent or latent HSV infection in a new cell type.

Work on identifying herpesvirus sequences in human autopsy tissues was extended to the study of arterial disease with and without association with VZV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01995-22 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Studies of Myelin Formation, Breakdown and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	H.deF. Webster, M.D.	Chief	LENP, NINDS
Others:	Q.-L. Zhang, M.D.	Visiting Fellow	LENP, NINDS
	P-X. Lin	Visiting Associate	LNC, NINDS

COOPERATING UNITS (if any)

Laboratory of Neurochemistry, NINDS

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.75	1.75	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to use tissue culture methods, quantitative light and electron microscopy, in situ hybridization, immunocytochemistry, and biochemical techniques to study cellular and molecular mechanisms of myelin formation, breakdown and regeneration. In nerve lesions involving injury to myelinated fibers, return of function depends on successful early interactions of regenerating axons with Schwann cells. Last year, we showed that supernatants prepared from proximal nerve segments 24-48 hours after transection significantly increased mitosis of cultured Schwann cells and also significantly increased their production of laminin, an extracellular matrix component that promotes outgrowth and elongation of regenerating axons after axotomy. When proximal nerve segments were bisected, supernatants prepared from both halves 24 hours after axotomy increased proliferation and laminin production of cultured Schwann cells. This year, we used ELISA assays, Northern blots, receptor binding assays and PCR to investigate the source and composition of the factor(s) in the proximal stump supernatants which are responsible for their early effects on Schwann cells located where nerve regeneration begins. Studies of the supernatants showed that the factor(s) were heat labile and probably were polypeptides of relatively low molecular weight. Assays, using specific antibodies, showed that supernatants prepared from both proximal and distal nerve segments had higher levels of calcitonin gene-related peptide (CGRP) and growth-associated protein 43 (GAP-43) than controls, but there was little difference in levels seen in proximal and distal segments, indicating that neither peptide was responsible for the effect we observed. Tests of other neuropeptides as well as other Schwann cell mitogens are in progress.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02550-13 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and Immunologic Mechanisms in Virally-Induced CNS Demyelination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS
Others:	H. Agostini, M.D.	Guest Researcher	LENP, NINDS
	K.E. Astrom, M.D.	Guest Researcher	LENP, NINDS
	G.S. Ault, Ph.D.	Sr.Staff Fellow	LENP, NINDS
	M. Ishaq, Ph.D.	Sr. Staff Fellow	LENP, NINDS
	H.G. Ressetar, Ph.D.	Sr. Staff Fellow	LENP, NINDS
	C. Ryschkewitsch, B.S.	Medical Technologist	LENP, NINDS
	H.deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS *(if any)*

Lab. Mol. Oncol., Alton Ochsner Med. Fdn. (O. Prakash); Dept. Mol. Biol., Penn State U. (R.J. Frisque); Neurol. Serv., VAMC West LA (E.J. Singer); Shirati Hospital, Tanzania (G. Brubaker)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.51	1.91	1.60

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews

☒ (b) Human tissues

☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns mechanisms of CNS demyelination in human disease and continues to focus on the human JC polyomavirus (JCV), the etiologic agent of progressive multifocal leukoencephalopathy (PML). PML is a fatal demyelinating disease which is the immediate cause of death in about 5% of AIDS cases, and can signal onset of clinical AIDS. Work this year has continued to emphasize the detection of the JC virus by polymerase chain reaction (PCR) in PML tissues and in human urine. In urine the other human polyomavirus, BK virus (BKV), is also found. Notable advances this year have involved: (1) detection of JCV and BKV in African urines obtained from AIDS patients in Shirati, Tanzania; (2) detection of mutated strains of JCV which are missing 24 or 27 nucleotides in the region of the capsid protein VP1; (3) characterization of demyelinated lesions in two cases of early PML discovered at autopsy; and (4) advances in understanding the possible role of astrocytes in the pathogenesis of PML. With the delineation of deletions in the VP1 coding sequence, we have now defined five potential molecular variables in JCV which might help to explain some of the clinical and pathological variables in this disease. These are: (1) the existence of two viral genotypes in the U.S.A., and a simple PCR typing method for determining the type of JCV infection; (2) existence of regulatory region variants due to re-arrangement within the host of an archetypal viral structure, a process which may prove to be the basis of JCV neurotropism; (3) regulation of small t antigen expression in non-AIDS and AIDS brain. Small t antigen is not essential for, but may enhance, JCV infection in the PML brain; (4) deletion of an epitope in VP1 (described above); (5) Mutations in the DNA-binding region of large T-antigen of a BK virus associated with end-stage renal disease in an AIDS patient (reported last year). Whether similarly virulent mutants of JCV exist or will arise is unknown, but the position of this mutation is such that it is predicted to influence the DNA binding of T-antigen to the viral origin and thus viral expression and replication.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02803-05 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Latency and Pathogenesis of Herpes Simplex Virus in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	W.J. Mitchell, D.V.M., Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand aspects of the pathogenesis of herpes simplex virus (HSV) infection in the nervous system including the mechanism by which this neurotropic virus is regulated within neuronal and non-neuronal cells during acute and long-term infections. A further objective is to understand the relationship between HSV infection and disease.

During FY 1994, studies to analyze previously developed transgenic mouse models of HSV-1 pathogenesis were continued. The primary goal was to investigate the mechanism by which HSV-1 immediate early gene expression and viral replication are regulated by specific host and viral transcriptional regulatory proteins during nervous system infections. Further analysis of transgenic mice containing the HSV-1 major immediate early promoter sequence fused to the *E. coli* beta galactosidase coding sequence has shown that specific subsets of neurons in the absence of viral proteins can activate the HSV-1 immediate early (ICP4) promoter. Thus, neurons contain the transcriptional regulatory proteins which are necessary to regulate the major immediate early promoter of HSV-1. Preliminary experiments have indicated that trigeminal ganglion neurons regulate the ICP4 promoter differently in newborn than adult mice. These observations are important to understand the mechanism of regulation of latent HSV-1 DNA in neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02804-05 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nervous System Regeneration in a Herpesvirus Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Prinicpal Investigator:	D.B. Henken, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS

COOPERATING UNITS *(if any)*

M.E. Goldstein, Ph.D., Bristol-Myer Squibb Pharaceuticals, Meriden , CT
R. Curtis, Ph.D., Regeneron Pharmaceuticals, Tarrytown, NY

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To understand better the long-term neurobiological interactions and effects of herpesvirus infection on host sensory ganglia, a mouse model has been developed in which evidence indicating neurite sprouting has been documented. At present we are determining whether these sprouts successfully regrow and reestablish connections in the CNS. If regeneration in this model can be demonstrated, then these studies, in addition to defining herpes-induced host neurobiological alterations, will provide new insight into the requirements for regeneration when compared with classical axon injury paradigms. Initial studies examine the effects of herpes simplex virus type-2 (HSV-2) infection on host dorsal root ganglia (DRG) following a peripheral footpad inoculation. This experimental model mimics many aspects of human clinical herpesvirus infection and may, in addition, provide insights into mechanisms of post-herpetic neuralgia.

During FY 1994, the following issues were addressed: (1) Can neurochemical alterations, that have been demonstrated in classical axotomy-induced regeneration models, be identified here? In a time-course study of HSV-2 infection, alterations of growth-associated protein (GAP-43), a marker usually identified with regeneration in neurons, was analyzed in DRG cell bodies and in their peripheral and central processes. (2) Can neurites be demonstrated in this model and do they contain GAP-43? Neurites have been observed incidentally in dorsal roots in another HSV model, but current studies aim to systematically examine this question at the ultrastructural level in this model. The primary findings were that in this model: (1) GAP-43 is increased in DRG, dorsal roots, and glial cells 2 weeks after inoculation. This result is further evidence that, following acute ganglionic HSV-2 infection, selective neurochemical alterations can be found in DRG neurons, and another indication that the molecules that are selectively induced may relate to neuronal regrowth. (2) Preliminary ultrastructural evidence indicates that neurites are present in the dorsal roots of HSV-infected mice in this model.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02900-01 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tumor Induction and JC Human Polyomavirus Infection in the Neonatal Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator	H.G. Ressetar, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Section Chief	LENP, NINDS
	H.deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study investigates cellular tropism of the human polyomavirus, JC virus (JCV) and the cellular events leading to induced brain pathology in humans and animal models of JCV infection. JCV remains latent following initial childhood infection, and reactivation of the virus in immunocompromised individuals causes the fatal CNS demyelinating disease, progress multifocal leukoencephalopathy (PML). When injected intracerebrally into newborn hamster, JCV establishes a nonproductive infection inducing brain tumor formation. In human PML, JCV infection of oligodendrocytes leads to cell death and subsequent demyelination. Astrocytes are also infected by JCV but the role of this cell in initial brain infection and disease progression is less well understood. Astrocytic infection by JCV as well as the possible involvement of other cell types in disease pathogenesis is being examined in: (1) human PML tissues, and (2) animal models of JCV brain infection.

Current efforts have focused on developing and improving immunostaining techniques to recover antigen expression in formalin-fixed paraffin-embedded PML brain tissue as well as devising double-label methods to identify JCV-infected cells in human and hamster brains. Improved staining sensitivity has revealed positive astrocytes in close association with blood vessels in PML brain. Additional viral expression was detected in GFAP negative perivascular cells. In contrast to these findings, JCV does not appear to infect astrocytes in the hamster model. Instead JCV primarily infects and induces neoplasia in granule neurons. Increased GFAP expression and reactive gliosis is evident in areas adjacent to neoplastic cells. In addition, JCV infects vascular endothelial cells in the hamster brain. These findings suggest that these cell types may possibly be involved in human disease and preliminary studies investigating endothelial cell involvement and blood-brain barrier alteration in the hamster model and human PML are in progress.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02808-05 LENP

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular and Molecular Studies of Growth Factors during Myelin Breakdown and Regeneration in the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator:	H. Webster , M.D.	Chief	LENP, NINDS
Others :	D. Yao, M.D.	Visiting Scientist	LENP, NINDS
	X. Liu, M.D.	Visiting Fellow	LENP, NINDS
	L. Hudson, Ph.D.	Staff Scientist	LVMP, NINDS
	C. Bondy, M.D.	Staff Scientist	DEB, NICHHD
	M. Brenner, Ph.D.	Staff Scientist	SB, NINDS
	J. Gehrmann, M.D.	Guest Researcher	LENP, NINDS

COOPERATING UNITS *(if any)*
Lab.of Viral and Molecular Pathogenesis DIR, NINDS; Developmental Endocrinology Branch, NICHHD; Stroke Branch, NINDS; Dept of Neuromorphometry, Max Planck Inst. Psych., Martinsried, Germany

LAB/BRANCH
Laboratory of Experimental Neuropathology

SECTION
Cellular Neuropathology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS 3.95	PROFESSIONAL: 3.95	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of the project are to study the glial gene expression of growth factors, myelin-related proteins and other glial proteins during nervous system injury and regeneration. In cuprizone intoxication and experimental autoimmune encephalomyelitis, there is demyelination, relative preservation of axons and myelin regeneration during clinical recovery. When focal cryogenic lesions are produced in dorsal columns of rat thoracic spinal cords, axons and myelin sheaths degenerate; glial cells and vessels survive and there is no hemorrhage or cavitation. During recovery, axons regrow and become remyelinated by Schwann cells and oligodendrocytes. Our recent studies have shown that in all three types of myelin injury associated with regeneration, hypertrophic reactive astrocytes in the lesions express the mRNAs and peptides for IGF-I and its binding protein IGFBP-2. As recovery and remyelination begin, the IGF-I receptor is expressed by oligodendroglia, the cells responsible for myelin regeneration. To test whether injury restricted to neurons produced the same astrocytic response, we examined astrocytic gene expression of GFAP and IGF-related proteins during retrograde degeneration of motor neurons following facial nerve transection. Compared to demyelination-induced astrocytic responses, neuronal injury induced less elevation of IGF-related protein gene expression by astrocytes. Finally, similar tests of IGF expression were carried out on rats with experimental autoimmune neuritis. This peripheral nerve disease produces brisk microglial cell activation in the spinal cord without inflammation or other cellular abnormalities. Although astrocytic expression of glial fibrillary acidic protein was increased, no increases in expression of IGF-I or IGFBP-2 were detected. This suggests that microglial cell activation alone does not have a major role in the induction of IGF-I gene expression during CNS injury.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02827-04 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification, Characterization, and Etiologic Role of Human Polyomavirus in Neurological Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator:	M. Ishaq, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS

COOPERATING UNITS *(if any)*

Dept. of Molecular and Cell Biology, Penn State University (R.J. Frisque)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews

☒ (b) Human tissues

☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

JC virus (JCV) causes a fatal demyelinating disease in AIDS patients known as progressive multifocal leukoencephalopathy (PML). This investigation was undertaken to understand the role of alternative T antigen splicing in the regulation of JCV replication in PML brain and the influence of HIV-1 on such regulation. An intron-differential RNA PCR was developed to study the expression of alternatively spliced JCV early mRNAs in PML patients with and without AIDS. The method utilizes primers which span the large T and small t antigen introns allowing amplification of specific cDNAs in the presence of contaminating viral genomic DNA. Hybridization with junctional probes and DNA sequence analysis confirmed the identity of the PCR products. The results show that JCV early mRNA is alternatively spliced as previously predicted by analogy to SV40. Large T antigen mRNA was detected in all the brain tissues from PML patients with and without AIDS. The expression of small t antigen mRNA varied depending upon the association of PML with AIDS and upon other unknown factors. Of the 12 PML/AIDS brain tissues, 11 (92%) expressed small t antigen mRNA, whereas only 8 of 13 (62%) with PML alone showed detectable levels of small t antigen mRNA. HIV-1 proviral DNA was detected in 10 of 12 PML/AIDS brains. The results indicate that alternative splicing of JCV early mRNA is regulated in the human brain, and that the production of small t antigen may not be essential for the pathogenesis of PML.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02847-03 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphological and Molecular Studies of CGRP in the Gastric Wall

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator:	G. Jakab, M.D.	Visiting Scientist	LENP, NINDS
Others:	E. Mezey, M.D.,	Visiting Scientist	CNB, NINDS
	K. Pacak, M.D.	Visiting Fellow	CNB, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[X] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated due to departure of Principal Investigator.

Publications:

Jakab G. Szallasi A, Agoston DV. The calcitonin gene-related peptide (CGRP) phenotype is expressed early and up-regulated by resiferatoxin (RTX) in mouse sensory neurons. Dev Brain Res 1994;-in press.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02849-03 LENP

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Human Polyomavirus Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator:	G.S. Ault, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Experimental Neuropathology

SECTION
Neurotoxicology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ubiquitous polyomavirus JC virus may be reactivated during prolonged immune suppression from a latent infection in kidney or other organs to a productive infection of oligodendrocytes known as PML. Events leading to brain pathology are not known but may include or result in alteration of the regulatory region. The kidneys of six PML patients were examined by PCR amplification with three primer pairs for the presence of JC virus and three were positive. The use of a PCR-based typing assay on all tissue samples, and cloned sequences from the viral coding region from each positive kidney, showed that the same viral genome was present in the kidney as in the brain of the patient. Regulatory region clones all had the archetypal promoter/enhancer structure. However, when PCR fragments from the regulatory region were digested with a restriction enzyme which cuts in region D, the region most often deleted in PML-type promoters, a low level of undigested DNA remained. This DNA refractory to digestion had a rearranged sequence identical to that of the uniquely rearranged promoter in the brain of each patient.

The archetype promoter/enhancer and three pro/enh obtained from PML brains representing increasing degrees of rearrangement of the archetypal pro/enh sequence were placed in front of the CAT reporter gene to assess transcriptional activity. In glial cells, the archetypal pro/enh is active in both early and late directions, and is comparable in strength to PML-derived regulatory regions. The moderately rearranged promoter, as well as the laboratory strain Mad-1 which is of the same rearrangement pattern, were the most efficient and showed the most stimulation of the late pro/enh by T-antigen. The minimally and highly rearranged regulatory regions were less efficient than the archetype. Differences in relative early to late promoter strength may be significant in JCV's ability to propagate in different cell types.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02882-02 LENP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Viruses as Vectors for Gene Transfer to the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS
Others:	S. Keir, Ph.D.	Visiting Fellow	LENP, NINDS
	W.J. Mitchell, D.V.M., Ph.D.	Sr. Staff Fellow	LENP, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been incorporated into Z01 NS 02549-13 LENP.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02884-02LMB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Cycle Regulation During *Drosophila* Visual System Development*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Brian Mozer, Ph.D.	IRTA	LMB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION
Development Biology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dissecting the mechanisms that regulate cell number during the development of the nervous system is of primary importance to understanding normal brain development as well as the aberrant control of cell growth that occurs in malignancies of the CNS. Genetic analysis of cell cycle regulation during Drosophila visual system development has identified the small eye mutant Drop (Dr) as a novel cell cycle control gene required for the initiation of eye morphogenesis. Developmental analysis of the dominant *Drop* eye imaginal disc phenotype suggests that the dominant alleles interfere with the expression of string (stg), the fly homolog of the yeast cell cycle regulator CDC25. The lack of anterior *string* expression in the mutant blocks the initiation and progression of the morphogenetic furrow resulting in a failure of photoreceptor cell differentiation leading to subsequent cell death.

Starting with a P[LacZ,w+] induced intragenic revertant of Dr^{Mio}, ~65 kb of genomic DNA from the distal 99A region of the third chromosome has been isolated containing part or all of the *Drop* gene. Analysis of the P-element insertion site revealed that the Dr^{Mio} chromosome contains a *3S18* retrotransposon not present in two different wild type strains. Insertion of the P-element into the *3S18* retrotransposon resulted in the complete suppression of the dominant small eye phenotype. Genomic Southern blot analysis of a collection of *Drop* mutations revealed that the dominant gain of function allele L2 and the loss of function alleles suD10, suD21, suD22, and suD61 are associated with deletions that remove part or all of the cloned region. Screening of an eye imaginal disc cDNA library has identified several candidate transcription units within the cloned interval. Experiments are currently in progress to determine: 1) which transcript encodes the *Drop* gene, 2) its pattern of expression in the eye disc and embryo, 3) the primary structure of the *Drop* gene product, and 4) the analysis of the loss of function phenotype of *Drop* with regard to mitotic control during embryonic and imaginal development.

*Formerly titled:
Identification of Genes that Specify Regional Differences in the Developing *Drosophila* Brain

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02881-02LMB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Biology of the Mammalian Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.D. McKay, Ph.D.	Acting Chief	LMB, NINDS	
Other:	J.P. Bengzon, M.D., Ph.D.	Visiting Fellow	R. Josephson	Pre-IRTA
	C.O. Brüstle, M.D., Ph.D.	Special Volunteer	Y. Maeda, M.D., Ph.D.	Visiting Associate
	D M. Collazo	Pre-IRTA	M.J. Marvin	Pre-IRTA
	L.M. Delgado-Rivera, Ph.D.	Visiting Fellow	S. Okabe, M.D., Ph.D.	Visiting Associate
	T.E. Hayes, Ph.D.	Senior Staff Fellow	G. Vaughn	Biologist
	T.G. Hazel, Ph.D.	IRTA	C. Vicario, Ph.D.	Visiting Fellow*

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
17.0	16.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The research program in this group is in the area of developmental neurobiology. The adult mammalian brain is composed of a vast number of different neurons. In embryonic development neurons are derived from multipotential precursor cells. When these precursors stop dividing they become committed to give specific neuronal types in a very precise pattern. This commitment step, which occurs in the few hours around the last division, controls critically important features of neuron numbers and types found in the adult brain. Our work is focused on the molecular and cellular mechanisms regulating this process.

The key methods we currently employ include: (1) the use of transgenic mice to define DNA sequences that target gene expression to neuronal precursors; (2) dissociated cell and tissue slice culture analysis of growth factors which regulate the proliferation, survival and differentiation of cells in the embryonic brain; and (3) the use of transplanted neuronal precursors to construct chimeric brains carrying genetically engineered functional neurons.

These techniques are used to analyze the molecular mechanisms controlling the development and function of the mammalian brain. The results are applicable to understanding the genetic basis of childhood tumors and neurodegenerative diseases of the central nervous system. They may also lead to powerful new therapies to reconstruct the damaged structure found in Parkinson's, Alzheimer's and Huntington's diseases.

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*Continued:

M. Dugich, Ph.D.	IRTA	T. Müller, Ph.D.	Visiting Fellow
T. Hisatsune, Ph.D.	Special Volunteer	D.M. Panchision, Ph.D.	IRTA
K. Johe, Ph.D.	IRTA	J.M. Pickel, Ph.D.	IRTA
U. Maskos, Ph.D.	Special Volunteer		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS 01309-29LMCN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Fishman, Ph.D.	Chief, Membrane Biochemistry Section	LMCN	NINDS
Others:	P.A. Orlandi, Ph.D.	Research Associate	LMCN	NINDS
	R. Ahmad	Special Volunteer	LMCN	NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cholera toxin (CT) produced by *Vibrio cholerae* and the heat-labile enterotoxin (LT) produced by certain strains of *Escherichia coli* are the causative agents of cholera and traveler's diarrhea, respectively. The toxins are structurally, immunologically and functionally similar: each has a pentameric B subunit which binds to specific receptors on the intestinal mucosal cell and an A subunit which is involved in activation of adenylylcyclase. Whereas CT uses the ganglioside GM_1 as its only receptor, LT appears to recognize G_{M1} as well as other receptors on intestinal cells. In order to identify these alternate LT receptors, we used human intestinal CaCo-2 cells, which behave in culture as differentiated enterocytes, the natural target for the two toxins. CaCo-2 cells bound 8-fold more LT than CT, and LT binding was only partially inhibited by CT-B whereas CT binding was completely inhibited by LT-B. Although G_{M1} was the only CaCo-2 glycolipid recognized by both CT and LT, a series of membrane galactoproteins (apparent molecular weights between 92 and 120 kDa) were recognized by LT but not by CT. By using specific glycosidases and transferrin and fetuin as model galactoproteins, we established that the binding determinant for LT on these galactoproteins was galactose (β1-4)N-acetylglucosamine. Furthermore, we were able to show that these galactoproteins recognized by LT were immunoprecipitated by specific antisera against polyactosylated glycoproteins of the lacto-N-neotetraosyl type. Finally, some of these alternate galactoprotein receptors for LT appear to be functional as activation of adenylylcyclase by LT in CaCo-2 cells was only partially inhibited by CT-B. Thus, despite the high degree of homology between LT and CT, LT can recognize glycoconjugates with carbohydrate structures distinct from the oligosaccharide of GM_1 which is recognized by both toxins. Furthermore, the apparent ability of LT to utilize both glycolipids and glycoproteins as receptors raises the possibility that LT may use more than one pathway for cellular activation. In this regard, we had shown previously that CT bound to neoganglioproteins enters the cell through a different pathway than when bound to G_{M1}.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS 02366-16LMCN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Receptor-Coupled Adenylylcyclase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Fishman, Ph.D.	Chief, Membrane Biochemistry Section	LMCN	NINDS
Others:	M.D. Pak, Ph.D.	Senior Staff Fellow	LMCN	NINDS
	X.-M. Zhou, M.D., Ph.D.	Visiting Associate	LMCN	NINDS
	Z. Wang, M.D., Ph.D.	Visiting Fellow	LMCN	NINDS
	P.K. Curran	Biologist	LMCN	NINDS
	D.E. Kauffman	Biologist	LMCN	NINDS
	Q.T. Hoang	Biologist	LMCN	NINDS

COOPERATING UNITS *(if any)*
Jesse Baumgold, Ph.D., Department of Radiology, The George Washington University Medical Center; Ronald S. Duman, Ph.D., Division of Molecular Psychiatry, Yale University School of Medicine

LAB/BRANCH
Laboratory of Molecular and Cellular Neurobiology

SECTION
Membrane Biochemistry Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 6.2	PROFESSIONAL: 3.2	OTHER: 3.0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to identify molecular mechanisms involved in the regulation of β-adrenergic receptor (βAR)-coupled adenylylcyclase. We previously reported that human β_1AR and β_2AR are regulated differently by agonists whether the receptors are endogenously expressed in human cell lines or stably expressed in transfected hamster cell lines. When exposed to agonist, the cells expressing β_2AR exhibit a rapid, typical pattern of desensitization of agonist-stimulated adenylylcyclase. Both maximum stimulation (V_{max}) is reduced and dose response (K_{act}) is shifted to lower sensitivity. By contrast, agonist-treated cells expressing β_1AR display a shift in K_{act}, but little or no reduction in V_{max}. It has been shown that desensitization of the β_2AR is mediated by protein kinase A (PKA) via phosphorylation of the third intracellular loop of the receptor and by the β-adrenergic receptor kinase (βARK) via phosphorylation of the C-terminus. By using specific kinase inhibitors, we have found that the reduction in V_{max} was mediated by βARK. This included the modest reduction observed for β_1AR. The shift in K_{act} was mediated by PKA. This was further confirmed by exposing the cells to a cAMP derivative. With cells expressing either subtype, there was a shift in K_{act}, but no reduction in V_{max}. These results indicated that human β_1AR was resistant to βARK-mediated desensitization. By contrast, hamster cells expressing rat β_1AR displayed a pattern of agonist-mediated desensitization similar to that of cells expressing human β_2AR. Whereas human β_1AR and β_2AR are only 54% homologous, the human and rat β_1AR are 91% homologous. Thus, only a small region of the receptor may be involved in βARK-mediated desensitization.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS02784-06LMCN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigation of Stimulatory Guanine Nucleotide Binding Protein Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.V. Rebois, Ph.D.	Unit Head	LMCN	NINDS
Others:	M. Toyoshige, M.D, Ph.D.	Visiting Fellow	LMCN	NINDS
	N.S. Basi, Ph.D.	IRTA Fellow	LMCN	NINDS
	D. Warner, Ph.D.	IRTA Fellow	LMCN	NINDS
	M. Nishimura, M.D., Ph.D.	Visiting Fellow	LMCN	NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular and Cellular Neurobiology

SECTION
Membrane Biochemistry Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.17	PROFESSIONAL: 4.17	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The stimulatory G protein (G_s) mediates activation of adenylylcyclase (AC). The α-subunit ($G_s\alpha$) of heterotrimeric (αβγ) G_s has a guanine nucleotide-binding site and intrinsic GTPase activity. Activation of AC occurs when an agonist-receptor complex promotes the exchange of GDP for GTP in the nucleotide binding site of $G_s\alpha$. Nucleotide exchange causes dissociation of Gsα from the βγ-subunit complex (Gβγ). $G_s\alpha$ then activates AC until GTP hydrolysis occurs and Gβγ reassociates with $G_s\alpha$. This model is widely accepted, and G_s subunit dissociation is a critical part of the model since Gβγ plays an important regulatory role in the process. However, we have recent experimental evidence to suggest that this model is incorrect. Bovine brain Gs activity was assayed by the ability to reconstitute AC in membranes from Gsα-deficient S49 cyc- (minus) cells. Gs subunit dissociation was assayed by immunoprecipitating Gsα and determining the amount of Gβ that was coprecipitated. Alternately, dissociation was measured by the ability of Gs to serve as a substrate for the bacterial toxin choleragen (CT), which catalyzes the ADP-ribosylation of heterotrimeric Gs, but not free Gsα. Magnesium ion caused a concentration-dependent dissociation of Gs in solution, and GDP and GTP were equally effective at inhibiting this effect. Activation of Gs with GTPγS, a nonhydrolyzable GTP analog, or with fluoroaluminate at physiological concentrations of magnesium ion, did not cause Gs subunit dissociation. Activation with fluoroaluminate caused Gs to dissociate in the presence but not in the absence of chloride ion. This was explained in earlier reports by others that fluoroaluminate caused Gs dissociation in the presence of low magnesium. Recombinant DNA techniques were used to construct a modified form of the 52 kDa version of Gsα which contained a 2.4 kDa peptide attached to the N-terminus (Gsα54.4). Gsα54.4 bound GTPγS and formed a heterotrimer with Gβγ in solution. When reconstituted into cyc- membranes, however, Gsα54.4 was unable to form a heterotrimer with Gβγ or activate AC unless the modifying peptide was removed by proteolysis. These data suggest that activated G_s heterotrimer and not the free Gsα-subunit mediates agonist stimulation of AC.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS01808-25LMCN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Glycoproteins of Myelin in Development and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard H. Quarles, Ph.D.	Section Chief	LMCN	NINDS	Jeffrey Hammer, Biologist
Others:	Sung Hye Yim, Ph.D.	Res. Biologist	LMCN	NINDS	
	Robert Farrer, Ph.D.	Sr. Staff Fellow	LMCN	NINDS	
	Zbigniew Bartoszewicz, Ph.D.	Visiting Associate	LMCN	NINDS	
	Naokazu Sasagasako, M.D.	Visiting Fellow	LMCN	NINDS	
	Carmen Pizzaro, Ph.D.	Special Volunteer	LMCN	NINDS	
	Carl Lauter	Chemist	LMCN	NINDS	

COOPERATING UNITS *(if any)*
Dept. Neurosciences, Cleveland Clinic Foundation, Cleveland, Ohio

LAB/BRANCH
Laboratory of Molecular and Cellular Neurobiology

SECTION
Myelin and Brain Development Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.2	PROFESSIONAL: 3.8	OTHER: 1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myelin-associated glycoprotein (MAG) is a member of the immunoglobulin gene superfamily that is localized in the periaxonal membranes of PNS and CNS myelin sheaths where it functions in glia-axon interactions and may be involved in transmitting signals from the axon to myelin forming cells. It occurs in two developmentally regulated isoforms with the large isoform, containing a longer cytoplasmic tail, predominating early in CNS development. The carbohydrate in MAG consists of a mixture of oligosaccharides, many of which are sialylated and sulfated, and which are currently being isolated and characterized. During this year, our studies on abnormalities of MAG expression in the dysmyelinating quaking mouse were completed. The results demonstrate a severe underexpression of the L-MAG isoform and several abnormalities of glycosylation, including increased sialylation, decreased content of the adhesion-related, HNK-1 epitope, and accumulation of a high mannose form of MAG. MAG expression in cultured oligodendrocytes and Schwann cells is being studied to determine factors that control its synthesis and posttranslational processing as well as investigating its function in intercellular adhesion and signaling. The immortalized Schwann cell lines generated in our laboratory express more MAG, MAG mRNA, P_0 glycoprotein, P_0 mRNA, sulfatide and galactocerebroside when their rate of growth is reduced by culturing in defined media or when the cells reach high density. The results suggest that these cells may be mimicking normal Schwann cells with regard to the necessity of down-regulating proliferation in order to differentiate toward myelination and should be useful for investigating factors controlling the expression of myelin constituents. Our previous findings, that GM3 ganglioside increases during normal oligodendrocyte differentiation in culture and that exogenous GM3 enhances the differentiation, were investigated further with regard to determining a possible effect of the ganglioside in modulating the activity of growth factor receptors. This year, we also examined an O-2A progenitor cell line (CG4) because of advantages that it may offer over primary cultures for investigating the functions of MAG and GM3. CG4 cells primarily express the L-MAG isoform and b-series gangliosides, and differ from primary oligodendrocytes by expressing very little GM3. The results suggest that the CG4 line will be useful for studying the functions of MAG and gangliosides in oligodendrocyte differentiation.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02786-06LMCN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antibodies to Glycoconjugates in Neurological Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard H. Quarles, Ph.D.	Section Chief	LMCN	NINDS
Others:	Robert Farrer, Ph.D.	Senior Staff Fellow	LMCN	NINDS
	Matthew Warden	Special Volunteer	LMCN	NINDS
	Carl Lauter	Chemist	LMCN	NINDS
	Jeffrey Hammer	Biologist	LMCN	NINDS

COOPERATING UNITS *(if any)*

Medical Neurology Branch, NINDS

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.6	PROFESSIONAL: 1.1	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This area of investigation began with the demonstration of monoclonal anti-MAG antibodies in patients with mixed sensory-motor polyneuropathies occurring in association with IgM gammopathy (paraproteinemia). It was subsequently demonstrated that these anti-MAG antibodies were all directed toward carbohydrate epitopes in MAG and cross-reacted with other glycoproteins of PNS myelin, including P_0 and PMP-22, as well as with the sphingoglycolipid, sulfate-3-glucuronyl paragloboside (SGPG). Monoclonal antibodies that are MAG/SGPG-negative in patients with gammopathy and neuropathy frequently react with ganglioside antigens in nerve. In the current year, we have demonstrated that a patient with a monoclonal IgAλ antibody and neuropathy is more complex than originally thought. Although, the patient has reactivity to LM1 ganglioside that is restricted to the IgA class, the reactivity is present in both IgA λ and κ light chains suggesting that it is polyclonal. Furthermore, the patient also has IgM reactivity of the MAG/SGPG type. Little is known about the molecular mechanisms by which antibodies to acidic glycolipids (SGPG or gangliosides) in patients with demyelinating neuropathy exert their pathogenic effects. In order to elucidate the functions of acidic glycolipids in Schwann cell differentiation and myelination, as well as to understand the mechanisms by which the human antiglycolipid antibodies may perturb function, we are investigating gangliosides in cultured Schwann cells. We have extended our previous results showing that culturing primary Schwann cells on a basement membrane substratum (Matrigel) resulted in greater synthesis of gangliosides (primarily GM3) and complex neutral sphingoglycolipids in association with increased cellular proliferation. Purified laminin and collagen type IV have effects comparable to matrigel, suggesting that these components may be the ones signaling the Schwann cell response. Furthermore, PDMP, an inhibitor of UDPglucose:ceramide glucosyl transferase, inhibited both the basement membrane-induced proliferation of Schwann cells and the synthesis of glycolipids, suggesting that this synthesis may be functionally involved in the proliferative response. Finally, an investigation of the effects Brefeldin A on glycolipids synthesis in an immortalized Schwann cell line suggests that the synthesis of sulfatide and hydroxy fatty acid-containing galactosylceramide occurs in the late Golgi, and that there may be different pathways for the synthesis galactosylceramides containing hydroxy and nonhydroxy fatty acids, respectively.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS02805-05LMCN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular and Immunological Aspects of Myelin Abnormalities in Neuro-AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Johanna R. Möller, M.D.	Unit Head, Senior Staff Fellow	LMCN	NINDS
Others:	Richard H. Quarles, Ph.D.	Laboratory Chief	LMCN	NINDS
	Jeffrey Hammer	Biologist	LMCN	NINDS
	Carl Lauter	Chemist	LMCN	NINDS

COOPERATING UNITS *(if any)*
Medical Neurology Branch, NINDS; Dept. of Neurology, Johns Hopkins University, Baltimore, MD; Cleveland Clinic Foundation, Cleveland, Ohio

LAB/BRANCH
Laboratory of Molecular and Cellular Neurobiology

SECTION
Demyelinating Disorders Unit, Section on Myelin and Brain Development

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.8	PROFESSIONAL: 0.6	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
  [ ] (a1) Minors
  [ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was undertaken to elucidate biochemical and immunological aspects of myelin disorders associated with neuro-AIDS. In the central nervous system these include diffuse myelin pallor (DMP) (decreased staining with Luxol fast blue), vacuolar myelopathy and multifocal demyelination. In the peripheral nervous system, this covers a demyelinating peripheral neuropathy. Postmortem subcortical white matter samples from AIDS patients with and without dementia were analyzed for quantitative and qualitative alterations of myelin proteins, including myelin-associated glycoprotein (MAG), myelin basic protein, proteolipid protein and 2',3'-cyclic nucleotide 3'-phosphodiesterase. The biochemical results were correlated with histological and immunocytochemical observations made by our collaborators at Johns Hopkins University on adjacent sections of tissue. DMP was detected histologically in about one-half of demented patients and one-fourth of the nondemented patients. However, electron microscopic, immunocytochemical and biochemical analyses of white matter indicated little or no loss of myelin proteins in areas of prominent DMP. On the other hand, substantial conversion of MAG to a proteolytic cleavage product (dMAG) was observed in some of the AIDS samples, as had previously been found in many samples from multiple sclerosis brain (see Z01 NS 02848-03 LMCN). Astrocytic hypertrophy was found in some of the AIDS patients both histologically and by increased levels of glial fibrillary acidic protein detected biochemically. Significant accumulation of serum proteins was detected immunocytochemically in white matter of many of the AIDS cases, especially the demented ones. This was supported biochemically by the presence of variable levels of haptoglobin on Western blots of AIDS samples but not of control samples. Overall, the results provide little evidence for significant demyelination or myelin pathology in subcortical white matter of AIDS brain, but suggest that blood brain barrier perturbation may contribute to CNS pathology in AIDS and AIDS dementia. The relationship of blood brain barrier breakdown to the proteolytic MAG/dMAG conversion observed in multiple sclerosis and AIDS brain is under investigation.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02848-03LMCN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Disorders of CNS Myelin

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Johanna R. Möller, M.D.	Unit Head, Sr. Staff Fellow	LMCN, NINDS
Others:	Richard H. Quarles, Ph.D.	Laboratory Chief	LMCN, NINDS
	Arun Chakrabarti, Ph.D.	Senior Staff Fellow	LMCN, NINDS
	Masayuki Sasaki, M.D.	Visiting Fellow	LMCN, NINDS
	Yukio Arai	Visiting Fellow	LMCN, NINDS
	Carl Lauter	Chemist	LMCN, NINDS
	Jeffrey Hammer	Biologist	LMCN, NINDS

COOPERATING UNITS (*if any*)
Developmental & Metabolic Neurology Branch, and Lab. of Exp. Neuropath., NINDS; School of Vet. Med., U. of Wisconsin; Pathology Dept., Michigan State U.; Neurology Dept., Kennedy Inst., Baltimore, MD.

LAB/BRANCH
Laboratory of Molecular and Cellular Neurobiology

SECTION
Demyelinating Disorders Unit, Section on Myelin and Brain Development

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects   [x] (b) Human tissues   [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Myelin basic protein (MBP) and proteolipid protein (PLP) are major proteins of compact CNS myelin, whereas myelin-associated glycoprotein (MAG) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) are mainly localized in associated oligodendroglial membranes. These 4 myelin proteins are differently affected in various dysmyelinating, demyelinating and remyelinating circumstances, and information about changes in them can increase our understanding of the specific molecular processes going on in each disease. In multiple sclerosis (MS), there is preferential loss of MAG at the edges of the plaques. Much of the MAG remaining in MS tissue is in the form of dMAG, a proteolytic cleavage product formed by a myelin-associated, calcium-activated neutral protease (calpain). MAG loss in MS may be related to this protease. dMAG was also present in some patients with neuro-AIDS (see Z01 NS 02805-05 LMCN). The MAG/dMAG conversion rate in incubated myelin, purified from different species, was greatest in human myelin, rapid in myelin from other primates, and substantially slower in myelin from lower mammals. This suggests that dMAG formation may be relevant to human demyelinating diseases. The MAG/dMAG conversion rate in purified myelin is very sensitive to the Ca^{2+} concentration in the samples and the levels of some gangliosides. Purified human calpain incubated with purified human MAG totally degraded MAG. In most hypomyelinating mutant animals, MBP and PLP are decreased more than MAG and CNP, due to a greater deficiency of compact myelin than of associated oligodendroglial membranes. This is true regardless of the primary cause of the hypomyelination, e.g. a PLP gene defect (Shaking pup), a cholesterol storage disorder (CSD mice), a congenital virus infection (Border disease in sheep) or in human patients with Niemann-Pick C disease. However, the TAIEP rat expressed decreased amounts of MAG compared to other myelin proteins, and MAG in the younger animals had a higher m.w. when compared to age-matched controls, most likely due to a glycosylation difference in the carbohydrate chains of MAG. In caprine β-mannosidosis, MAG, CNP and PLP levels were equally decreased, but MBP was relatively spared. This might be due to the presence of large storage vesicles which interfere with protein transport of MAG, PLP and CNP, while MBP translation is at the site of insertion into the myelin. Biochemical and histologic analysis of white matter biopsies from 2 young girls with a progressive leukodystrophy due to unknown causes, revealed the presence of all the characteristic myelin proteins and lipids, but at significantly decreased amounts.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS02864-03LMCN

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dopaminergic, Neurotrophic Factors and Reinnervation of the Spinal Cord

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John W. Commissiong, Ph.D.	Unit Head	NTU, LMCN	NINDS
Others:	Takao Takeshima, M.D., Ph.D.	Vis. Fellow	NTU, LMCN	NINDS
	Jane M. Johnston, Ph.D.	Vis. Fellow	NTU, LMCN	NINDS
	Helen Balling, B.S.	Biologist	NTU, LMCN	NINDS

COOPERATING UNITS *(if any)*
Medical Neurology Branch, NINDS

LAB/BRANCH
Laboratory of Molecular and Cellular Neurobiology, BNP

SECTION
Neural Transplantation Unit (NTU)

INSTITUTE AND LOCATION
Park Building, NINDS, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.25	PROFESSIONAL: 2.25	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have scaled up the production of type-1 astrocytes, and after partial purification based on ion exchange chromatography followed by gel filtration, identified two glycosylated proteins of molecular mass 15 kD and 50 kD that retain activity for promoting survival of fetal, dopaminergic neurons in culture. We will also attempt to purify the potent dopaminergic, neurotrophic factor present in the conditioned medium from O-2A progenitor cells. The O-2A progenitors can be easily expanded in culture in protein-free, defined medium from a relatively small number of E16 striatal cells, using bFGF (10 ng/ml) as the mitogen, versus ventral mesencephalon or cerebral cortex of the same age. A reasonable working hypothesis is that an O-2A-like cell might be present in the striatum, and could be the source of the target (striatum)-derived dopaminergic neurotrophic factor we have identified in the conditioned medium. Regulating production of this putative neurotrophic factor is of considerable physiological importance, since it could provide a mechanistic explanation of the cause of idiopathic Parkinson's disease (PD). We have tested the three major monoamines, all at 1.0 μM, for their effect on survival of striatal O-2A progenitors in culture. Dopamine (DA) was severely toxic, norepinephrine (NE) had no effect, and 5-hydroxytryptamine (5-HT) caused marked increased survival of O-2A progenitors. We are currently trying to determine if 5-HT is mitogenic, or whether it acts by promoting survival of O-2A progenitors generated under the influence of bFGF. The function of the dense 5-HT innervation of the striatum is presently unknown. Our preliminary results suggest that it might be involved in regulating production of a dopaminergic neurotrophic factor. Dopaminergic neurons are present in the midbrain, diencephalon, olfactory bulb, retina, spinal cord and dorsal root ganglia. Of these, the highly organized nigrostriatal, DA system is the one that is most severely compromised in PD. Idiopathic PD should now be regarded as a neurotrophic deficiency disease caused by reduced production of a target-derived, dopaminergic neurotrophic factor. We have standardized a bioassay method to test dopaminergic neurotrophic activity reliably, based on the use of microcultures and an imaging method. B49 neuroblastoma cells are the source of GDNF, the most potent DA neurotrophic factor isolated to date. Conditioned medium from O-2A progenitors contains much more DA neurotrophic factor than that from type-1 astrocytes which in turn contain more than that from B49 neuroblastoma cells.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01983-23 LMMN*

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Molecular Pathogenesis of JC Virus-Induced Progressive Multifocal Leukoencephalopathy

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

P.I.:	Eugene O. Major, Ph.D.	Chief	LMMN
Others:	Walter Atwood, Ph.D.	Sr. Staff Fellow	LMMN
	Blanche Curfman, B.S.	Microbiologist	LMMN
	Linda Durham, M.S.	Biologist	LMMN
	Rene Traub, B.S.	Microbiologist	LMMN
	Kei Amemiya, Ph.D.	Crada, Special Volunteer, Igen, Inc.	
	Toshiya Shinohara, Ph.D.	Special Volunteer	LMMN

COOPERATING UNITS (*if any*)

AIDS Clinical Trial Group, OAR, OD, NIH, Animal Health Care Section, Washington, Veterans Administration Hospital, D.C., Dept. of Neurology, George-August-Universitat-Gottingen, Germany

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Section on Molecular Medicine and Virology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.50	2.05	2.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The presence of JCV in peripheral blood lymphocytes (PBLs) in both viral induced progressive multifocal leukoencephalopathy (PML) and non-PML patients has strengthened the concept that JCV is carried to the brain by a hematogenous route. At the same time, it has focused a part of our studies to develop a quantitative analysis of the level of JCV in these cells and other tissues. It may be important in helping to assess the risk of non-PML patients and to evaluate the affect nucleotide analogs for the treatment of PML. Noncompetitive and competitive or quantitative PCR (QPCR) analysis procedures are being developed to ascertain the level of JCV present in different tissues. Control JCV DNA templates are being constructed to serve as external or internal standards for the quantitation of the virus. These procedures utilize the same primer set used to detect JCV in patient samples by PCR analysis which eliminates the possible differential hybridization kinetics when using different primer sets. A rapid and sensitive chemiluminescent procedure is being used to analyze the PCR products in these studies. An examination of the nucleotide sequences present in the prototype (Mad1) and brain-type (Mad 8B) JCV is being made to assess the role of these sequences in the pathogenicity of JCV. One major difference is the presence of a 23bp sequence which is present in most strains of JCV isolated from brain biopsies from PML patients. This 23bp sequence has a putative binding site for the transcription factor SP1. However, we have found that SP1 binds to this sequence very weakly as seen by competitive binding and DNase 1 protection assays. In addition, construction of CAT reporter plasmids containing either the Mad1 or Mad8B regulatory region has shown that the Mad1 sequence is much more active that that from Mad8B. Further studies are underway to understand the role of the 23-bp sequence in the expression of the Mad8B strain in different tissues.

*Formerly in LVMP.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS 02852-03 LMMN*

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neurogenesis and Gliogenesis in the Developing Human Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Eugene O. Major, Ph.D.	Chief	LMMN
Others:	Kei Amemiya, Ph.D.	Crada, Igen, Inc.	
	Walter Atwood, Ph.D.	Senior Staff Fellow	LMMN
	Peter Kennedy, M.D., Ph.D.	NIH Scholar	
	Joline Staehli	Special Volunteer	LMMN
	Charlotte Sumner	Howard Hughes Scholar	LMMN
	Rene Traub, B.S.	Microbiologist	LMMN

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Medicine and Neuroscience

SECTION
Section on Molecular Medicine and Virology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.15	PROFESSIONAL: 1.50	OTHER: 0.65

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of the common association between the binding sites of the NF-1 and AP-1 transcription factors in the regulatory region of a number of genes, we callthe adjacent binding sites for these two factors the "glial neurobox". It has been suggested that the NF-1 site in the JCV regulatory region is required for both JCV early transcription and DNA replication. Because of the possible involvement of NF-1 with the expression of JCV and other genes expressed in the CNS/PNS, we have screened cDNA libraries prepared from neonatal and fetal brain tissue for the possible presence of a brain specific NF-1 factor with an oligonucleotide probe homologous with the DNA binding domain of the NF-1 cDNA clone reported from HeLa cells. A number of c-DNA clones were isolated from both cDNA libraries. Two of the clones from the neonatal brain library and one clone from the fetal brain library were sequenced. The 3 sequenced c-DNA clones were homologous with each other, except for a few bases at the 5' end of the two longest clones and a 150-bp insertion in the 3'-region of the shortest clone. The DNA binding region located at the 5' -end of the clones was highly conserved between the brain clones and that reported for the HeLa NF-1 clone. On the other hand, the 3'-region of the c-DNA clones isolated from the brain libraries were highly homologous but differed from that reported from HeLa cells. The 3'-region of NF-1 molecules were reported to contain the transcriptional activational domain of the molecule. One of the brain cDNA clones was cloned into a T7 RNA polymerase expression vector, and a specific size protein was over-produced on induction of expression with IPTG. An extract prepared from cells overproducing the specific protein was demonstrated to contain a specific binding activity with an NF-1 oligonucleotide. In order to determine if expression of other classes if the NF-1 family could be detected in primary fetal brain cells. RT-PCR analysis was performed with poly A-selected RNA from primary fetal cells and HeLa cells. With the use of class-specific primers the expression of at least four classes of the NF-1 protein family could be detected in both the brain and the HeLa cell lines. Similar studies were performed with the class specific primers with RNA extracted from B-cell lines, however, without the detection of any PCR products. These results suggest that the expression of processing of NF-1 genes(s) in B-cells is different than that in the brain and HeLa cell lines, since specific NF-1 binding activity was detected by both competitive binding and DNase 1 protection assays with extracts prepared from B-cells.

*Formerly in LVMP.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02851-03 LMMN*

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
HIV-1 Infection in Human Fetal Brain Cell Cultures and Pediatric AIDS Brain Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Eugene O. Major, Ph.D.	Chief, LMMN
Others:	Walter Atwood, Ph.D.	Senior Staff Fellow, LMMN
	Gene Brashears	Biologist, LMMN
	Katherine Conant, M.D.	Senior Staff Fellow, LMMN
	Maneth Gravell, Ph.D.	Microbiologist, LMMN
	Chiara Monaco, Ph.D.	Guest Worker, Bologna, Italy

COOPERATING UNITS *(if any)*
A. Facchini, Univ. Bologna, Italy; A. Degrassi, Udine Univ Sch Med, Italy; S. Houff, VA Hosp., Washinton, D.C.; J. Berger, Univ. Miami Sch. Med., Miami; R. Youle, SNB, NINDS; F. Chiodi, Karolinska Inst., Sweden

LAB/BRANCH
Laboratory of Molecular Medicine and Neuroscience

SECTION
Section on Molecular Medicine and Virology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.13	PROFESSIONAL: 3.33	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
 ☐ (a1) Minors
 ☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human astrocytes can be infected with HIV-1 both *in vivo* and *in vitro*. However, the amount of HIV-1 structural and nonstructural protein production is low compared to that of the macrophage or the microglial cell in the brain. In an *in vitro* model using human fetal astrocytes, several weeks following infection or transfection, cocultivation with uninfected lymphocytes or stimulation with the cytokines TNF-α and IL1-β will increase viral production from this cell type. In the current work we have demonstrated that phorbol 12-myristate 13-acetate (PMA) also increases HIV-1 p24 production from the primary human astrocyte. Using electrophoretic mobility shift assay (EMSA) in combination with supershift studies using specific antibodies, we demonstrated that PMA, like TNF-α increases the p50/p65 form of NF-KB. Furthermore, we also showed that the protein kinase inhibitor H7 inhibits PMA and TNF-α associated increases in HIV-1 expression at a time when it has little to no inhibitory effect on the associated increases in p50/p65 NF-kB. Thus, unless p50/p65 NF-kB or its binding is affected by H7 in a manner that cannot be resolved by EMSA, an increase in this form of NF-kB is not always sufficient to increase HIV-1 expression from the astrocyte. We have also shown *in vivo* infection of astrocytes with HIV-1 expressing the nonstructural protein, *nef*. This observation was confirmed independently by other investigators in pediatric cases. The role of the infected astrocyte in the pathogenesis of HIV-1-associated encephalopathy con-tinues to be studied. Also, HIV-1 appears to establish a persistent infection in the stromal cells of the lymphoid tissues. The similarities of this infection to that of the astrocyte is being studied at the molecular level targeting NF-kB induction and binding to the HIV-1 LTR.

The Laboratory is also investigating the ability of specific RNases to inhibit the multiplication of HIV-1 in lymphocyte cell lines. Onconase and bovine seminal RNase were shown to block infection of HIV-1 in productivity infected cell lines. This block appears from an intracellular mechanism of RNase activity.

*Formerly in LVMP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS 02904-01 LMMN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Molecular and Cellular Neurologic Therapeutics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Carlo S. Tornatore, M.D.	Acting Section Chief	LMMN
Others:	Belinda Baker, Ph.D.	Irta Fellow	LMMN
	Rebecca Hamilton, B.S.	Biologist	LMMN
	Karen Meyers	Biol. Lab. Tech.	LMMN

COOPERATING UNITS *(if any)*
Clinical Neurosciences Branch, DIR, NINDS

LAB/BRANCH
Laboratory of Molecular Medicine and Neuroscience

SECTION
Molecular Therapeutics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects
  [ ] (a1) Minors
  [ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Molecular Therapeutics Section was established in January 1994 to develop novel therapeutic approaches to viral and neurodegenerative diseases of the CNS. The approaches to be taken by this Section are outlined as follows:

1) Development and Transplantation of Neuroglial Cell Lines: An immortalized human fetal astrocyte cell line previously developed in this Laboratory has been successfully grafted into the basal ganglia of Sprague-Dawley rats and the subhuman primate, rhesus macaque. No tumor formation or obvious inflammatory response was seen. Experiments are being initiated in which MPTP-lesioned macaques would have this immortalized human fetal astrocyte cell line grafted to the basal ganglia in an attempt to reverse the parkinsonian symptoms by induction of neuronal sprouting. Efforts will also be directed towards understanding the immune response to these xenografts and the implications for human transplantation.

2) Development of a Novel Viral Vector for Gene Transfer into the CNS: Delivery of genes to selected cell populations within the CNS could potentially be used as a means for targeted delivery of therapeutic agents. Given that JC virus expression is restricted in the brain to cells of glial origin, we have constructed a novel chimeric JCV which expresses a cloned gene of interest in cells of glial origin but fails to replicate. This chimera has the same host range as wild type JCV and consequently could be used to deliver antiviral compounds to JCV infected glial cells in a patient with PML.

3) Development of Immunotherapy Targeted Against Viral Diseases of the CNS: Experiments are now in progress to determine whether we can initiate an immune response that will clear JCV from the CNS by expressing noninfectious viral antigens in the skeletal muscle of a patient with PML. A plasmid capable of expression of JCV structural antigens in skeletal muscle of a patient with PML. A plasmid capable of expression of JCV structural antigens in skeletal muscle has been constructed and is being tested *in vitro*. *In vivo* experiments are to follow.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02791-06 LMMN*

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Replication and Pathogenesis of Enveloped Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Schubert, Ph.D.	Section Chief	LMMN
Others:	S.-Y. Paik, Ph.D.	Visiting Fellow	LMMN
	C.-J. Chen, Ph.D.	Visiting Associate	LMMN
	A.C. Banerjea, Ph.D.	Senior Staff Fellow	LMMN
	B. Lewis	Lab Technician	LMMN

COOPERATING UNITS *(if any)*
K. Ozato, Laboratory of Molecular Growth Regulation, NICHD, NIH, Bethesda, MD. C.Y. Kang, Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada

LAB/BRANCH
Laboratory of Molecular Medicine and Neurosciences

SECTION
Molecular and Viral Genetics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.45	PROFESSIONAL: 1.15	OTHER: 0.30

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects [ ] (b) Human tissues [x] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year this study continued to focus on the role of the viral matrix protein M of vesicular stomatitis virus (VSV) in cytopathogenesis and in viral assembly. We had previously reported that the VSV M protein is responsible for cytopathic effects which involve the disorganization of the cytoskeleton after VSV infections. Other investigators also found that the matrix protein, which remains the only protein of this cytoplasmic virus found in cell nuclei is able to inhibit cellular transcription. We have studied this property of the M protein after isolation of HIV-1 susceptible cell clones which encode either a wild type or a temperature sensitive M protein under the control of the inducible HIV-1 LTR promoter. Cell clones which encode this protein were infected with HIV-1 as well as with HIV-2. Gene expression and replication of both viruses were drastically inhibited by wild type M protein after transactivation with the HIV Tat1 and Tat2 proteins, respectively. Although HIV-1 was able to establish a provirus in these cells at the same efficiency as in cells without M protein, subsequent viral gene expression and spread were drastically inhibited in M expressing cell lines. Since basal levels of wild type VSV M protein were not toxic to the cell, it appeared to function as an inducible antiviral. The mechanism for the inhibition of cellular transcription by the VSV M protein was further studied in the context of interferon inducible genes which are turned off after VSV infections. A novel nuclear DNA binding protein was detected in a gel shift assay after VSV infections. Binding was detected with a specific interferon stimulated response element sequence. In collaboration with Dr. Ozato's group, we found that the induction of this protein binding was dependent on VSV gene expression and replication. With one of our temperature M protein mutants, this binding was temperature sensitive, which indicated that the VSV M protein may play a role in that shift. Supershift experiments did not identify M protein as part of the DNA/protein complex itself. Further experiments are in progress to examine the potential role of M protein in inducing this new DNA binding activity as well as its role in the shut off of cellular transcription. These studies will allow important insights into the mechanism of VSV induced cytopathogenesis as well as into specific mechanisms of gene regulation, possibly involving new negative cellular transcription factors.

*Formerly in LVMP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02818-05 LMMN*

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pseudotypic Defective Interfering HIV Particles as an Antiviral Therapy for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	M. Schubert, Ph.D.	Section Chief	LMMN
Others:	S-Y. Paik, Ph.D.	Visiting Fellow	LMMN
	G.G. Harmison II, M.S.	Chemist	LMMN
	A.C. Banerjea, Ph.D.	Senior Staff Fellow	LMMN
	C.-J. Chen, Ph.D.	Visiting Associate	LMMN
	B. Lewis	Lab.Technician	LMMN

COOPERATING UNITS (if any)

A. Perelson and G. Nelson, Theoretical Biol. & Biophys. Group, Los Alamos Natl. Lab., Los Alamos, NM

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Molecular and Viral Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.95	0.30

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
  [ ] (a1) Minors
  [ ] (a2) Interviews

[ ] (b) Human tissues

[X] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have proposed an antiviral strategy against the human immunodeficiency virus (HIV-1) which employs a defective interfering HIV-1 particle that can be targeted to HIV-1 infected cells. Expression of this defective virus would interfere with HIV-1 replication and, at the same time, use the structural and regulatory proteins of HIV-1 for its own replication and spread throughout the population of HIV-1-infected cells. Toward the generation of such defective interfering HIV-1 particles, we have isolated a stable cell line which encodes a complete copy of one of our recombinant defective interfering HIV-1 proviruses. This provirus encodes a chimeric CD4/Env protein as well as a multitarget-ribozyme under control of the HIV-1 LTR. These cells could be infected by HIV-1 as efficiently as cells without the defective provirus. This was shown by HIV-1 proviral integration at 10 hr postinfection. Challenge of the cells by HIV-1 infection resulted in a transactivation of gene expression from the LTR of the defective provirus. As compared to the cell line which does not contain the defective interfering provirus, the amount of spliced and unspliced mRNAs from the superinfecting wild type virus was drastically reduced. The amount of infectious virus released from these cells was reduced up to 300 fold, as were syncytia formation and the amounts of p24 antigen in cell supernatants. The infectivity of the released virus was also reduced 2- to 4-fold as a result of a change in the make-up of the released virus, which seemed to contain less viral envelope protein. Polyadenylated defective interfering HIV-1 RNA was packaged into virus particles, which opens the possibility for a transfer of the interfering genes through HIV-1 like particles in the future. Challenge of the same cells with HIV-2 only resulted in an approximately 2-fold reduction in the virus yield. We conclude that the main mechanism of HIV-1 inhibition in this cell line was caused by the multitarget-ribozymes encoded by the defective provirus and the resultant sequence-specific degradation of HIV-1 transcripts. These results demonstrate that inducible, defective interfering HIV-1 proviruses can be very efficient in an intracellular immunization against HIV-1.

*Formerly in LVMP.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02908-01 LMMN

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Gene Delivery to Nondividing Cells of the CNS

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

P.I.	M. Schubert, Ph.D.	Section Chief	LMMN
Others:	G.G. Harmison, M.S.	Chemist	LMMN
	S.-Y. Paik, Ph.D., Ph.D.	Visiting Fellow	LMMN
	A.C. Banerjea, Ph.D.	Sr. Staff Fellow	LMMN
	B. Lewis	Lab. Technician	LMMN

COOPERATING UNITS (*if any*)
S. Karlsson, Section Chief and J. Reiser, Visiting Scientist, DMNB, NINDS, Bethesda, MD

LAB/BRANCH
Laboratory of Molecular Medicine and Neuroscience

SECTION
Molecular andViral Genetics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.65	PROFESSIONAL: 0.50	OTHER: 0.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The purpose of this study is to evaluate the potential use of a defective human immunodeficiency virus (HIV-1) as an expression vector for nondividing cells, and in particular for cells of the central nervous system (CNS). The project addresses several virological elements which must be functional for a gene expression vector, to allow the targeted and stable expression of genes in differentiated, nondividing cells. Such a vector would obviously be very beneficial for many areas of the life sciences. It would also have a potential therapeutic use for several genetic diseases. HIV-1, a retrovirus and a member of the lentivirus family, presents itself as a potential candidate for such a vector: As a characteristic of this virus family, its members are able to translocate their preintegration complexes through the nuclear membrane, which is essential for proviral integration.The project has recently been initiated, and we have completed the assembly of several DNA constructs which will be used to generate and select HIV-1 packaging cell lines for the expression of the structural and regulatory proteins of HIV-1. DNAs which encode several different viral envelope proteins, including murine leukemia virus ecotropic and amphotropic glycoproteins as well as the vesicular stomatitis virus glycoprotein have been cotransfected with HIV-1 and packaging constructs. Studies are in progress to quantitate defective HIV-1 pseudotype viruses within the cell supernatants by stable transformation. Subsequent studies will focus on the efficiency of proviral integration into nondividing cells, packaging of foreign RNA, viral targeting and stable gene expression. We anticipate that the basic knowledge about these functions on the molecular level, will enable us to develop novel defective virus particles with the described properties for the gene delivery and expression in the CNS.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02907-01 LMMN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Multitarget-Ribozymes as Analytical and Therapeutic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M. Schubert, Ph.D.	Section Chief	MVGS, LMMN
Others:	C.-J. Chen, Ph.D.	Visiting Associate	MVGS, LMMN
	B. Lewis	Lab Technician	MVGS, LMMN
	J. Harms	Volunteer	MVGS, LMMN

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Molecular and Viral Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.75	0.40	0.35

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have initially developed the first multitarget-ribozymes in the context of an antiviral strategy against the human immunodeficiency virus (HIV-1). Their antiviral activity was very effective and specific to justify an entire project dedicated to the development and application of these catalytic RNAs not only against viruses but also against cellular RNAs.

We had previously determined that multitarget-ribozymes can be synthesized which catalytically cleave target RNA very efficiently at multiple sites in vitro, functioning simultaneously like several restriction enzymes for RNA. This multiple sequence specificity was also demonstrated by us in vivo after HIV-1 infections. Most importantly, we had found that at the same molar ratio, multitarget-ribozymes are much more effective in RNA cleavage than single ribozymes. In addition, multitarget-ribozymes seemed to retain most of their activity even when they are buried within a large RNA transcript. These features could be very attractive, for example, in protein replacement experiments or even in therapies. These results also suggested that multitarget-ribozymes could also be used to eliminate the expression of genes in transgenic animals without requiring homologous recombination after appropriate localized delivery to differentiated cells in vivo possibly through viral vectors.

Towards these goals, we need to understand the precise mechanism of ribozyme action, its intracellular site of action, how efficient target sites can be selected, etc. We anticipate that these studies will lead us to new analytical tools for the study of cell function and possible treatment of cellular dysfunctions of the central nervous system as well as other organs. This project has only been initiated recently and, although we have assembled several DNAs encoding mutated multitarget-ribozymes, we do not have a definite answer as yet in which cellular compartment ribozymes are active and whether multitarget-ribozymes can in part also function as antisense molecules.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01686-26 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Motor Control Systems in the Spinal Cord

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.E. Burke, M.D.	Chief	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	G.M. Dold	Engineering Tech.	LNLC, NINDS
	E. Simon, M.D.	Staff Fellow	LNLC, NINDS
	A. Degtyarenko, Ph.D., D.Sc.	Visiting Associate	LNLC, NINDS
	S. Schiff, M.D., Ph.D.	Neurosurgeon	Children's Hosp.

COOPERATING UNITS *(if any)*
Dept. of Neurosurgery, Children's Hospital National Medical Center, Washington, DC (Dr.Schiff)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neural Mechanisms

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.76	2.4	0.36

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
The project is designed to provide information about the organization of neuronal systems in the mammalian spinal cord that are involved in motor control, as studied in adult cats *in vivo* and in the isolated brain stem and spinal cord of neonatal rats or mice studied *in vitro*. Current interest centers on the organization of excitatory last-order interneurons in reflex pathways within the spinal segment and control of information flow in them by input from primary afferent and supraspinal descending systems, and their interaction with the spinal mechanisms that generate rhythmic motoneuron output patterns underlying locomotion. In the cat, we are particularly concerned with interneurons that transmit short-latency excitation from low-threshold skin afferents and from reticulospinal and vestibulospinal systems that all produce minimally disynaptic excitation in some species of lumbosacral motoneurons. All these interneuron groups are strongly influenced by the spinal central pattern generator (CPG) for locomotion. The differential patterns of CPG modulation indicate that separate systems of segmental interneurons, each with highly specific patterns of primary afferent and descending convergence, are present in the mammalian spinal cord. Work on the *in vitro* preparation of neonatal rodent spinal cord, with or without the brain stem, is in its early stage and is aimed at developing preparations that can be used to investigate functionally defined reflex pathways, in order to compliment work in the cat. We hope to develop techniques suitable to study neural systems activated by particular peripheral nerves and descending systems, with extracellular recording from identified peripheral nerves and intracellular recording from select populations of motoneurons and interneurons. Early results have been encouraging and promise to provide a means to study specific neural systems under conditions that are impossible to achieve in the *in vivo* situation.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01687-26 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Techniques for Making Connections with the Nervous and Musculoskeletal Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.J. Bak	Electronics Engineer	LNLC, NINDS
Others:	R.E. Burke, M.D.	Chief	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	F.T. Hambrecht, M.D.	Health Scientist Administrator	DFN, NINDS
	M.J. O'Donovan, M.B., Ch.B.	Section Chief	LNLC, NINDS
	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS

COOPERATING UNITS *(if any)*
Instrumentation and Computer Section, BNP, DIR, NINDS (G.R. Dold)

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous systems in acute and chronic neurophysiological preparations. Because of this Laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable microelectrodes, mechanical transducers, catheters, and connectors.

Due to the Laboratory's new interests in doing research on isolated preparations such as the spinal cord of chicken embryos, a significant amount of work has been devoted to improving techniques associated with electrical recording, stimulation, and real-time fluorescence microscopy in these preparations. Also included within this report is the development of computer programs of general utility for acquisition and analysis of neuroelectric and mechanical records, as well as of neuroanatomical material.

Several projects which have been associated with the visual prosthesis feasibility studies, normally reported on under this project number, are now being reported under project, Z01 NS 02857-02 LNLC. These projects are referenced as such throughout this report. There are also several other new projects which would normally be listed under this project number but are now listed under the recent visual prosthesis project number.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01688-26 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cortical Mechanisms of Voluntary Motor Control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	D. Cole	Biologist	LNLD, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	F. Faruque	Student Volunteer	Johns Hopkins Univ.
	W.J.Heetderks, M.D., Ph.D.	Health Scientist Administrator	DFN, NINDS

COOPERATING UNITS *(if any)*
Fundamental Neuroscience Program, NINDS (W.J.Heetderks); University of Michigan (K.Wise)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neural Mechanisms

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	0.4	1.4

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multicontact passive semiconductor electrodes, supplied by the University of Michigan, have been successfully implanted in the arm area of the supplementary motor cortex of a primate that was trained to do a number of different wrist movement tasks. The activated iridium recording sites were discovered to have impedance characteristics that were a function of a DC bias that was applied to the electrodes. Many times, when the electrode was in the high impedance state, neuronal activity was close to the noise level of the recording system, or nonexistent. Switching the electrode to the low impedance state lowered the noise of the recording system and revealed neuronal activity. A single pulse of less than 0.1 microamperes is sufficient to shift the electrode to the low impedance state. By using this pulse and biasing technique, we have been able to extend the recording time of the semiconductor electrodes to over eight months of chronic recording.

Regrowth of connective tissue at the implant site was seen to break some of the silicon ribbon cables that were an integral part of the electrode. A new electrode and connector design has been developed that should overcome the breakage problem.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02079-21 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Models of Neurophysiological Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.B. Marks, Ph.D.	Research Physiologist	LNLC, NINDS
Others:	R.E. Burke, M.D.	Chief	LNLC, NINDS
	M.J. O'Donovan, M.B.Ch.B., Ph.D.	Research Physiologist	LNLC, NINDS
	T.G. Smith, Ph.D.	Research Physiologist	LNP, NINDS

COOPERATING UNITS *(if any)*
Lab. of Neurophysiology, NINDS (T.G.Smith)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neural Mechanisms

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In previous Annual Reports, we described various approaches to summarizing the shape of neuronal dendrites, including a stochastic algorithm with empirical parameters that produces realistic individual dendrites and extensions that compute averages of these. This year we have searched for physiological functions of shape which increase for shapes more closely resembling natural dendrites. If such a function is found, it would suggest a "goal" for the dendrite. We have tried ratios of an estimate of total synaptic input, to dendrite volume used as a measure of metabolic cost. Inputs were weighted by local coupling: the fraction of any injected current that reaches the soma. When input was assumed proportional to coupling-weighted surface area, the access function grew without limit for dendrites with unnatural bulbs at large distances, but not when this was prevented by requiring natural taper, so natural taper was adopted as a constraint. These access functions can grow unnaturally large for large local surface areas, useless for synapses, because they saturate the local extracellular volume. This convinced us to include an explicit estimate of extracellular volume accessed. Local volumes within a distance R of the dendrite were weighted by the electrotonic coupling to the soma of the dendrite nearpoint, and summed. This summed, weighted volume was normalized by dendrite volume. The ratio is greater for branching structures, greater for dendrites which branch more near the soma, a property of motoneurons, and greater when the structures have approximately natural extent. This access function gives a meaning to ideas like "optimum stem diameter" and other natural optimum features.

With Dr Smith we have made progress in utilizing "wavelets", an alternative to Fourier transforms, as a mathematical tool to express salient features of cultured neurons and glia.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02160-20 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Intrinsic Properties of Motor Units

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	R.E. Burke, M.D.	Chief	LNLC, NINDS
Others:	W.B. Marks, Ph.D.	Research Physiologist	LNLC, NINDS
	B. Ulfhake, M.D., Ph.D.		Karolinska Institutet
	R.E.W. Fyffe		Wright State
	W.Cameron		Univ.Pittsburgh

COORPERATING UNITS (*if any*)
Lab. of Neurophysiology, NINDS (T.G.Smith)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neural Mechanisms

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	0.4

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project is designed to provide information about the populations of motor units that make up large limb muscles in mammals. The scope of work includes studies of the electrophysiological and morphological characteristics of spinal cord motoneurons, the organization of synaptic inputs to them, and the relationship of these central nervous system factors to the mechanical, histochemical and anatomic properties of the muscle fibers (termed "muscle units") innervated by the motoneurons. Current work on this project is largely focused on neuroanatomic studies and computer modeling of individual, functionally-identified motoneurons, with emphasis on the fundamental features that control dendritic morphology and the influence of dendritic anatomy on the electrical properties of neurons and mechanisms of information processing in dendrites. We have devised a relatively simple stochastic model that is useful to isolate the key factors that control dendritic morphology. This approach is being used to compare the fundamental dendritic structure of several groups of cat motoneurons, as well as the morphologies of motoneuron dendrites in two groups of motoneurons during postnatal development. We are using the original data and computer-generated dendrites to explore the electrophysiological consequences of different dendritic structures. We are now extending these studies to examine the quantitative characteristics of dendritic trees in three dimensions.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02254-18 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Repair of Injured Nervous Tissue with Foreign Grafts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.A. Zalewski, M.D.	Medical Officer	LNLC, NINDS
Others:	N.A. Azzam, Ph.D.	Biologist	LNLC, NINDS
	R.N. Azzam	Biologist	LNLC, NINDS
	J.D. Ziemnowicz	NIH Special Volunteer	LNLC, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neuronal Regeneration

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	1.0	1.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
A short course of immunosuppressive therapy with Cyclosporin A (Cy-A) can induce immunological tolerance in rats to kidney and heart allografts. In contrast, continuous Cy-A treatment was needed to promote nerve allograft survival. Indeed, after stopping Cy-A, nerve rejection occurred and host axons that regenerated into the graft disappeared despite the fact that they are not foreign tissue and should not be the target of an immune response. Treatment of the graft rather than the recipient could be another way to induce tolerance. If the cell type(s) responsible for inciting the immune response could be removed from a nerve prior to its transplantation, tolerance to the nerve might develop. Intravascular blood cells and/or connective tissue cells could be the cause of nerve allograft rejection. Accordingly, the following treatments (alone or in combination) were used to remove these cells from 4-cm-long ACI rat nerves prior to their transplantation into Fischer (FR) hosts: whole body perfusion with a physiological salt solution, whole body irradiation (1000 Rads from a cobalt source), systemic antilymphocyte serum therapy. After receiving the treatments cited, some nerves were desheathed of epineurium and perineurium and used as endoneurial allografts. Finally, ACI nerves, resident for 5 months in Cy-A-treated FR hosts, were retransplanted into new, untreated FR rats. This experiment addressed the question of whether a physiological turnover of endoneurial macrophages could alter nerve allograft immunogenicity. None of the treatments used induced tolerance to nerve allografts when they were examined 4 weeks postoperatively. All the allografts were infiltrated by mononuclear cells and Schwann, vascular and perineurial cells were absent. These results indicated that Schwann and/or vascular cells are immunogenic components of nerve. Because rat strains may differ in their response to short-term Cy-A therapy, we plan to examine the fate of nerve allografts in the original strain combinations (PVG and DA rats) where tolerance to kidney and heart allografts was described. Experiments will be performed to determine if host axonal loss can be prevented during nerve allograft rejection caused by the withdrawal of Cy-A. This might be possible since anti-inflammatory agents have been reported to decrease axonal loss caused by inflammation that develops after acute spinal cord injury. Reports that some host axons can regenerate through short lengths (2 cm) of rejected nerves prompted us to evaluate this method of nerve repair. In contrast to surviving nerve allografts, nerve segments formed after rejection had fewer axons, more neuromas, and no permeability barriers. These results should raise concern about using nerve allografts to repair nerves in nonimmunosuppressed recipients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02787-06 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Network Function in the Developing Spinal Cord of the Chick Embryo

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.J. O'Donovan, M.B., Ch.B., Ph.D.	Reseearch Physiologist	LNLC, NINDS
Others:	N. Chub, Ph.D.	Visiting Fellow	LNLC, NINDS
	A. Donevan, Ph.D.	Guest Researcher	LNLC, NINDS
	W.B. Marks, Ph.D.	Research Physiologist	LNLC, NINDS
	A.M. Ritter, Ph.D.	Guest Researcher	LNLC, NINDS

COOPERATING UNITS *(if any)*
Dept. Physiol., Yale University, New Haven, CT (L. Cohen); Dept. Anat., Hebrew Univ., Jerusalem, Israel (A. Lev-Tov)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Developmental Neurobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.85	4.50	1.35

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
The project is concerned with analyzing the development and function of spinal networks in the spinal cord of the chick embryo. One focus is the synaptic organization of the lumbosacral cord. A second interest is in the cellular and network mechanisms responsible for the genesis of spontaneous network activity. A third area is in establishing the function of spontaneous neural activity during development with particular reference to the control of gene expression. All experiments are performed on an isolated preparation of the spinal cord which is maintained *in vitro*.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02788-06 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Neuronal Shape

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.L. Smith, Ph.D.	Senior Staff Fellow	LNLC, NINDS
Others:	J. Drazba,Ph.D.	IRTA Fellow	LN, NINDS
	V. Lemmon, Ph.D.		Case West. Res. U.
	E. Bearer, Ph.D.		Brown U.

COOPERATING UNITS *(if any)*
Case Western Reserve University (V. Lemmon); Brown University (E.Bearer)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Developmental Neurobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project uses structural methods to study neurons grown *in vitro* with the goal of understanding the molecular mechanisms involved in neurite outgrowth and pathfinding. One initiative focuses on the initial outgrowth of neurites from isolated chick peripheral ganglion neurons, using time-lapse video microscopy with conventional and laser scanning microscopes. Neurite formation begins when a filopodium is invaded by a bulge of perinuclear cytoplasm, which contains microtubules, neurofilaments and organelles and is surrounded by a cortex of actin microfilaments. These cytoplasmic components invade the filopodium, converting it into a definitive neurite with a growth cone at its distal end. Experiments in which neurons were grown in the presence of drugs that inhibit the dynamic turnover of microtubules showed that the initial steps in neurite formation do not require assembly of microtubules and provided evidence that microtubules assembled in the cell body can be translocated into neurites as they emerge.These findings were used to formulate a model of the cytoskeletal mechanisms and substrate interactions that lead to the initiation of neurite outgrowth. Current work tests predictions of this model. A second initiative concerns mechanisms of growth cone migration. In order for a growth cone to pull itself across a substrate, its actin cytoskeleton must become linked to molecules in the plasma membrane that mediate adhesion to that substrate. Several proteins that fibroblasts use to link their actin cytoskeleton to the substrate at focal contacts have been studied using immunofluorescence techniques to determine whether these proteins aggregate in growth cones at sites of contact with the substrate, as identified by interference reflection microscopy. Knowing the identity of the linkage proteins and how their interactions with actin filaments are regulated is essential to understanding growth cone migration.

(This project will be transfered to the Laboratory of Molecular Biology, BNP, DIR, NINDS in FY 1995.)

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02857-03 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Feasibility Study of an Intracortical Visual Prosthetic Device for the Blind

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLD, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	A. Reina	Summer Student	LNLC, NINDS

COOPERATING UNITS *(if any)*
Fundamental Neurosciences Program, NINDS (W.J. Heetderks and F.T. Hambrecht); Surgical Neurology Branch, NINDS (C.V. Kufta); Instrumentation and Computer Section, BNP, DIR, NINDS(B. Smith, Chief)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neural Mechanisms

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project is designed to evaluate the feasibility of a visual prosthesis for totally blind individuals by stimulating chronically implanted microelectrodes in the visual cortex. As reported last year, a 42 year old woman who has been blind for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensations of light called phosphenes. The results suggest that it may be possible to produce a useful visual prosthesis if more electrodes are implanted. A new protocol for implanting up to 256 microelectrodes in the visual cortex has been approved by the Institute's Human Review Board. A 256-channel, microprocessor-controlled stimulation system has been coonstructed and is now undergoing final evaluation prior to testing with animals. A camera interface that will allow stimulation of the electrode array with visual images is nearing completion. A new laser system can remove Parylene-C insulation from the electrodes and provide a much better tip exposure than has been possible with any other technique. The new head-mounted, multicontact connector, developed in conjunction with PI Medical, is undergoing final bench testing before animal implantation. The next patient should be implanted during FY 1995.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02899-01 LNLC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neural Mechanisms Controlling Breathing in Mammals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: J.C. Smith, Ph.D. Research Physiologist LNLC, NINDS
Others:

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neural Mechanisms

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.55	0.55	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to provide information on basic neural mechanisms involved in the generation and control of respiratory movements in mammals. The long-range goal is to explain the ontogeny and neurogenesis of respiratory movements in terms of the biophysical, synaptic, and network properties of respiratory neurons in the mammalian brainstem and spinal cord. Current work focuses on cellular and network mechanisms generating the respiratory rhythm in the brainstem. A set of interrelated, multidisciplinary studies are ongoing to determine: sites, cellular components, and architecture of brainstem networks involved in generation and transmission of respiratory rhythm; biophysical properties and synaptic interactions of rhythm-generating neurons; and neurochemical mechanisms for modulation and synaptic transmission of rhythm. Experiments are performed with isolated *in vitro* brainstem-spinal cord and brainstem slice preparations from fetal, neonatal, and juvenile rodents. The critical brainstem locus containing the populations of neurons generating the rhythm has been identified. Novel *in vitro* slice preparations containing this critical region and functionally active respiratory networks have been developed which provide a powerful experimental approach for analysis of mechanisms concurrently at cellular and network levels. Computational approaches are being used in parallel to experimental studies to model respiratory neurons and networks. Computational models of the respiratory oscillator have been developed which are allowing analysis of cellular and network mechanisms that are currently difficult to study experimentally.

(The principal investigator has recently arrived and has not yet begun actual work on the project. A full project report will be made in FY 1995.)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01442-28 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Permeability of Cellular Layers in the Vertebrate Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Bechara Kachar, M.D.	Visiting Scientist	LNO, NIDCD

COOPERATING UNITS *(if any)*

Marine Biological Laboratory, Woods Hole, MA; Woo Kuen-Lo, Ph.D., Dept of Anatomy, Morehouse Medical School, Atlanta, GA.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

How tight junctions might prevent small charged solutes from entering the brain (across the blood-brain barrier) was made clear by our previous model of tight junction structure based on a lipidic backbone. This model has been discussed and elaborated in several publications in recent years. A new study of the gap junctions in murine cells by freeze-fracture and freeze-substitution has been completed showing that these gap junctions show considerable structural diversity, falling into there types. One type is so aberrant from typical gap junction structure as to question its categorization as an electrotonic junction. This work has now been published and this project in now in abeyance.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01881-24 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Basis of Synaptic Transmission

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Jorge E. Moreira, Ph.D.	Visiting Scientist	LN, NINDS
	Katsuyuki Miyaguchi, M.D.	Visiting Associate	LN, NINDS

COOPERATING UNITS *(if any)*

R.Llinas, P.M. Reuss. Dept of Physiology and Biophysics, New York Univ Medical Center, NY

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.35	0.75	0.60

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project deploys a range of structural techniques to examine normal synaptic structure. These approaches have in common their dependence on rapid freezing and direct visualization of living brain by light microscopic techniques. Up until now this project has been engaged in explorations of various live brain preparations suitable for these purposes. Recently, an isolated whole brain preparation maintained *in vitro* by vascular perfusion as well superfusion with artificial cerebrospinal fluid (CSF) as been evaluated. The ultrastructure of surface samples of isolated brains rapidly excised, quick frozen and freeze-substituted served as a benchmark to choose a perfusion fixative yielding realistic images of synaptic structures. It could now be determined to what extent deeper cortical regions of perfused brains remained structurally intact. Throughout 2 hours of perfusion, the morphology of synaptic structures in the isolated brain remained equivalent to the normal brain perfused-fixed *in situ*. These results provide an excellent method for structural work on the isolated brain, and show that the isolated brain can be used for studies of synaptic structure depending on rapid freeze fixation, and are in agreement with the reported persistence of electrophysiological functions in this preparation. (A comprehensive effort to develop methods for making and maintaining organotypic brain cultures continues and now appears to be successful - Project Z01 NS 02610-11 LN). Thus, these results will be reported but the main future emphasis will be on organotypic cultures.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02551-13 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Cytoplasmic Motors*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Mark Terasaki, Ph.D.	Senior Staff Fellow	LN, NINDS
	Shahid Khan, Ph.D.	Guest Researcher	LN, NINDS
	Alexandra Schmidek	Summer IRTA	LN, NINDS

COOPERATING UNITS *(if any)*

Elaine Bearer, Ph.D., Department of Pathology, Brown University; R.D. Leapman, Ph.D, BEIP, NCCR, NIH, Bethesda, MD; B.J. Schnapp, Ph.D., Dept. Cell. Mol. Physiol., Harvard Medical. School., Boston, MA.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.55	PROFESSIONAL: 0.90	OTHER: 0.65

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to understand the distribution and functions of cytoplasmic motors in the axon of neurons. This information is intended to lead to an understanding, at the molecular level, of fast and slow axonal transport as well as the cytoplasmic organization in the axon. The central project in the Section on Structural Cell Biology, has been particularly active this year, providing a plethora of new findings. A new functional assay for myosins was introduced and used to uncover a novel class of myosin II-like motors on the surfaces of squid axonal organelles. This 235 kDa myosin is double headed and appears to adhere strongly to the surfaces of axonal organelles. The organization of the actin substrates for these motors in the axon is also under study. A possible assay useful for approaching the difficult problem of defining the mechanism of slow transport has also been introduced. Macromolecular assemblies injected into the squid giant axon move in the anterograde direction only and require ATP. Of particular interest is that short actin filaments also move anterogradely and that all movements appear to be along some type of intracellular tract. This in vitro assay may make it possible to define the motors for such movements. The bacterial flagellar motor in E. coli has been studied as another example of a motor system than can switch direction of translocation. We recently discovered a new cytoplasmic component of the flagellar motor thought to be involved in directional switching-antibodies to known switching components appear to stain this new structure.

*Formerly entitled: "Proteins Involved in Axonal Transport and Structure of Neuronal Cytoplasm"

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02873-03 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunocytochemistry of Neuronal Cytoplasm

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge E. Moreira, Ph.D.	Visiting Scientist	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Sven Beushausen, Ph.D.	Visiting Associate	LN, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.75	PROFESSIONAL: 0.75	OTHER: .0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antibodies to defined domains of the light and heavy chains of the motor protein kinesin (from squid axon) have been used for immunolabeling of freeze-substituted squid axoplasm. It was necessary to develop and apply cryogenic methods to prevent displacement of soluble kinesin during tissue processing. We found that kinesins are widely distributed in the cytoplasm but several-fold concentrated around cytoplasmic organelles. The higher concentration is on vesicles (69.6-fold), but increase in gold particles over the cytoplasmic level was also seen around endoplasmic reticulum (ER) cisternae (29.5-fold), microtubules (15.2-fold), and mitochondria (6.2-fold). New experiments using affinity-purified polyclonal antibodies against a defined protein fragment of the functional head of the kinesin heavy chain confirmed the previous kinesin location. Western blots performed with the same antibodies, using pure squid kinesin, whole axoplasm, and pellet and supernatant of the centrifuged axoplasm, confirmed the results showing the 116 Kd kinesin band on all the samples. Sections incubated with a polyclonal antibody against squid neurofilament, failed, as expected, to show a selective distribution around vesicles. Preliminary results using antibodies which distinguish different kinesin light chain isoforms detected similar gold distribution as shown for the heavy chain but comparatively higher on the cytosol and lower on organelles. Hindrance due to the larger volume of the heavy chain as the light chain attaches to organelles it may explain the lower label on organelles and higher label of free kinesin on the cytosol. Further work is needed for the interpretation of these results. Processing of this data is expected to suggest whether the differences are due to different exposition of the kinesin light chain on organelles or cytosol. This work is expected to elucidate where different members of the kinesin family are found in the axon, leading to a better understanding of how kinesins actually function in the nerve cell.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS02835-04 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Subcellular Organization in Excitable Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Evelyn Ralston, Ph.D.	Special Expert	LN, NINDS
Others:	Stefanie Kaech, Ph.D.	Visiting Fellow	LN, NINDS
	Thorkil Ploug, M.D.	Special Volunteer	EDMN, NIDDK
	Sven Beushausen, Ph.D.	Visiting Associate	LN, NINDS
	Bernhard E. Flucher, Ph.D.	Visiting Associate	LN, NINDS
	Thomas S. Reese, M.D.	Chief	LN, NINDS

COOPERATING UNITS *(if any)*
Jill Horowitz, Div. Hematol. Product, CDER, FDA

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.75	PROFESSIONAL: 1.75	OTHER: 1.00

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects [ ] (b) Human tissues [x] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to understand how mRNAs, proteins, and subcellular organelles are distributed and organized in muscle and nerve cells. In multinucleated muscle cells, the nonuniform distribution of specific mRNAs and proteins contributes to the formation of the neuromuscular junction. In neurons, differential distribution of specific mRNAs may play a role in the establishment and maintenance of axonal-dendritic polarity. We are trying to understand how mRNA stability and mRNA translation influence mRNA localization. To that effect, we are localizing, by *in situ* hybridization, both endogenous and foreign mRNAs from transfected genes, in the mouse muscle cell line C2, and in primary cultures of rat hippocampal neurons. In the past year, we have focused our work on the endogenous transferrin receptor (TfR). It was found that at both mRNA and protein levels, distribution of TfR mRNA is not uniform in myotubes. Localization of TfR mRNA appeared independent of iron concentration in the medium, a modulator of TfR mRNA stability, indicating that distribution of TfR mRNA is independent of its half-life. On the other hand, treatment with protein synthesis inhibitors suggested that attachment to ribosomes on the rough endoplasmic reticulum is, at least in part, responsible for localization of TfR mRNA. We will now try to identify sequences responsible for this localization by transfection of chimeric TfR cDNAs. We have shown that TfR mRNA in neurons is confined to the cell body and will now determine if translation is part of this localization. An important advance with the neuron work has been the development of a modified protocol for transfection of neurons with the lipid reagent DOTAP, resulting in a five-fold increase in transfection efficiency. We are continuing to investigate the mechanism of vesicle and protein traffic in muscle by studying the localization of the glucose transporter GLUT4 and its translocation to the plasma membrane following stimulation by insulin or exercise. An important result was to find, contrary to previous reports, that C2 cells express GLUT4 as part of their normal differentiation program. Young myotubes appear unable to translocate GLUT4 to the cell surface, but mature myotubes undergoing spontaneous contraction, appear capable of doing so. We are presently investigating the mechanism that regulates GLUT4 translocation in C2 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02872-03 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Cell Adhesion in Vertebrate Neural Development

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Judith A. Drazba, Ph.D.	IRTA Fellow	LN, NINDS
Others:	Stefanie Kaech, Ph.D.	Visiting Fellow	LN, NINDS

COOPERATING UNITS (*if any*)

Vance Lemmon, Ph.D., Department of Neuroscience, Case Western Reserve University, Cleveland, OH.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.00	PROFESSIONAL: 1.00	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to characterize the role that cell adhesion molecules play in the development of neuronal polarity. Neurons produce two types of processes, axons and dendrites. Molecules have been identified, such as axonal L1, that localize to one or the other of these processes. It is not known how this differential distribution develops, nor whether it is the result or the cause of the morphological differentiation in these cells. To address this question we investigated whether L1 could influence the mechanisms that underlie axonal or dendritic development. We plated embryonic rat hippocampal cells onto substrate-bound L1, allowed the cells to develop for varying periods of time (a few hours to a few weeks), and then fixed and immunostained them for polarity markers L1, MAP2, and tau. We found that the cells extended multiple, extremely long axons within 12 hours. These axons continued to elongate rapidly without fasciculating and branched in atypical patterns. Dendritic development was significantly suppressed for at least a week. In comparison, sister cultures on poly-L-lysine required at least 24 hours for short axons to develop, but elaborated dendrites shortly thereafter. The cell adhesion molecule, N-cadherin, which is more uniformly distributed over the surface of mature neurons, also affected process outgrowth when used as a substrate. It greatly, and uniformly accelerated the growth of both axons and dendrites. Cells plated on the extracellular matrix component, laminin, showed a selective increase only in axonal growth over that seen on poly-L-lysine. This is consistent with previously published reports. The differential distribution of polarity markers, such as axonal L1, and the dendritic microtubule associated proteins MAP2 and tau, correlated with the accelerated development of polarity. We are continuing to characterize the development of neurons growing on L1 and N-cadherin, looking at other differentially distributed molecules and performing quantitative analyses of process numbers and lengths. We are analyzing the distribution of cytoskeletal elements such as actin and tubulin, and are evaluating the role of protein kinases. This may help us to elucidate the observed effects since both of these are believed to be involved in adhesion molecule signaling. We are also using interference reflection microscopy to visualize the interaction patterns of axonal and dendritic growth cones with various adhesion molecules. This may reveal differences in levels or patterns of adhesion that underlie the effects of these molecules.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02871-03 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Postsynaptic Densities: Mechanisms for Structural Modification

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ayse Dosemeci, Ph.D.	Visiting Associate	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS

COOPERATING UNITS *(if any)*
Howard Jaffee, Ph.D., Protein/Peptide Sequencing Facility, LNC, NINDS, NIH

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.05	PROFESSIONAL: 1.05	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium-dependent changes in postsynaptic densities (PSDs) may be involved in activity-dependent modification of synaptic function. The most abundant protein in PSDs is similar or identical to the alpha-subunit of calcium/calmodulin-dependent protein kinase II. We studied the autophosphorylation characteristics of the associated calcium /calmodulin-dependent protein kinase (CaM kinase) in isolated PSDs. When endogenous phosphatase is inhibited, the PSD-associated kinase, like its cytosolic counterpart, autophosphorylates in the presence of calcium, or in the absence of calcium, following brief incubation with the ion. Sequencing of phosphopeptides indicates that a major site of phosphorylation under both conditions is a threonine residue corresponding to T-253 of the alpha-CaM kinase II sequence. This mode of regulation is unique to the PSD-associated enzyme, since the soluble CaM kinase is described to be autophosphorylated at this site only at low concentrations of ATP and only in the presence of calcium. Differences in phosphopeptide patterns generated from the kinase autophosphorylated in the presence and absence of calcium indicate the presence of additional sites autophosphorylated under one condition only. Thus, PSD-associated CaM kinase, through its intricate, multisite autophosphorylation properties, may be capable of recording fluctuations of calcium. Studies on structural changes in PSDs mediated by another calcium-dependent enzyme, calpain, were continued. Electron microscopy of single and serial sections from freeze-substituted samples indicate that, following limited proteolysis that caused extensive degradation of spectrin but left most other major components intact, the central lamina of the PSD looked less dense and widened, as if it had unraveled. It is likely that these changes are due to breakdown of spectrin-mediated cross-bridges. Such a mechanism might serve to expose occluded sites in PSDs.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02841-04 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of the Endoplasmic Reticulum

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark Terasaki, Ph.D.	Senior Staff Fellow	LN, NINDS
Others:	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Jorge E. Moreira, Ph.D.	Visiting Scientist	LN, NINDS
	Thomas S. Reese, M.D.	Chief	LN, NINDS

COOPERATING UNITS *(if any)*
L.Jaffe, A. Fein, Univ. Conn. Health Ctr., Farmington, CT; S.L. Tamm,Boston Univ. Mar. Prog.; N.T. Slater, Northwest. Univ. Med. Sch.; C.Sardet, CNRS, Villefranche, France; J.Lippincott-Schwartz, NICHD, NIH.

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.35	PROFESSIONAL: 1.35	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure and function of the endoplasmic reticulum (ER) in neurons and glia are being investigated using newly developed techniques which make it possible to investigate the dynamic properties of the ER in living cells by video and laser scanning confocal microscopy. New techniques have been developed using sea urchin eggs as a model system. Sea urchin eggs have the advantages of being readily available, having a prominent ER, and being easy to microinject. A novel technique for specifically staining ER in living cells shows that the ER undergoes actin-dependent movements, that a striking change in its organization occurs as it becomes capable of releasing calcium at the time of calcium release, and that the appearance of microtubules has a profound effect on its organization. This technique has been applied to Purkinje neurons of the cerebellum in acute slices, where it shows that there is a continuous compartment of ER that extends from the cell body throughout the dendritic tree. The structure and dynamics of the ER is currently being investigated in hippocampal neurons in culture, where the ER may have a role in the establishment of axonal and dendritic polarity. A second initiative uses calcium-sensitive fluorescent indicators to investigate calcium regulation by the ER. Calcium increase during ciliary reversal has been detected, and calcium regulation in neurons in frog tadpoles is being investigated with these techniques.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02842-04 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Neural Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sven A. Beushausen, Ph.D.	Visiting Associate	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Stefanie Kaech, Ph.D.	Visiting Fellow	LN, NINDS
	James Willard, Ph.D.	IRTA Fellow	LN, NINDS
	Harish Pant, Ph. D.	Research Chemist	LNC, NINDS
	Ellen Meier, Ph. D.	Senior Staff Fellow	LVMP, NINDS
	Heinz Arnheiter, M.D.	Visiting Scientist	LVMP, NINDS

COOPERATING UNITS (if any)

H. Jaffe, Ph.D. Protein/Peptide Facility, LNC, NINDS; K. R. Weiss, Ph. D., W. Probst, Ph. D., D. Applegate, Dept. Physiol. Biophys., Mt. Sinai Sch. Med.,NY; S. Augustine, Dept. Neurobiol. Duke Univ., NC

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.75	PROFESSIONAL: 1.75	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The following summary describes five projects that attempt to investigate, at the molecular level, the roles of a number of molecules including, kinesin light chains, rab 3, p67 a novel activator of neuronal Cdk5, the modulatory neuropeptides buccalin and myomodulin and acidic calponin, play in neural function. Kinesin light chains, rab 3 and p67 are proteins that either directly or indirectly affect synaptic vesicle transport or fusion. We are currently attempting to address protein function by mutation and knock-out analysis by homologous recombination with kinesin light chains and p67 or by protein expression and microinjection in the case of rab3. We have previously characterized the peptide families of buccalin and myomodulin from the sea hare *Aplysia californica* and are currently examining how they collectively contribute to pre- or postsynaptic neuromodulation. The molecular characterization of receptors, the signal transduction cascades that are activated as a consequence of binding and the target proteins downstream of activation are also actively being pursued. Acidic calponin is a 36 kDa novel isoform of the actin binding protein calponin. Immunofluorescence studies using peptide specific antisera to its unique C-terminal tail domain have localized the protein to areas of high membrane turnover, particularly, the cleavage furrow of dividing cells, growth cones of neurons and sites of injury. Further research will be directed towards elucidating its role in wound healing and nerve regeneration.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02897-01 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synaptic Plasticity and Development in Organotypic Cultures of Hippocampus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Katsuyuki Miyaguchi, Ph.D.	Visiting Associate	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Judith A. Drazba, Ph.D.	IRTA Fellow	LN, NINDS
	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
	Ayse Dosemeci, Ph.D.	Visiting Associate	LN, NINDS

COOPERATING UNITS *(if any)*

Carlos Collin, Ph.D., Neural Systems Section, NINDS, NIH

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.20	PROFESSIONAL: 1.20	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project employs a range of structural and biochemical techniques to examine normal synaptic plasticity and development. These approaches have in common their dependence on rapid-freezing and direct visualization of living brain by light microscopic techniques. This project has been engaged in exploring various live brain preparations suitable for these purposes. Recently, success has been achieved with a new approach to culturing hippocampal slices. Their typical laminar organization and most of their thickness can be maintained for up to 12 weeks by culturing them at the interface between air and culture medium. The slices also maintain their normal electrophysiological function. Now, ultra-structural studies of the slices are being carried out on cultures of different ages using both chemically fixed and freeze-substituted preparations. Preliminary observations indicate modifications of synaptic morphology in long-term cultured slices. After five weeks in culture, many concave-shaped spines appeared, and some had "spinules", finger-like indentations of the postsynaptic membrane projecting into the presynaptic terminal. After nine weeks in culture, the number of concave-shaped spines and spinule-containing spines decreased, but the presynaptic terminal enlarged and many segmented synaptic active zones appeared. These structural changes are reminiscent of previously reported activity-dependent changes in synaptic morphology. Taken together, these results show that hippocampal organotypic cultures, prepared according to our new method, will be an excellent preparation to examine synaptic plasticity and development.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02610-11 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Elemental and Structural Organization of Neurons and Glia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Asher Shainberg, Ph.D.	Guest Researcher	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS *(if any)*
R.D. Leapman, BEIP, NCCR, NIH; D. Landis, Case-West.Res. Univ, Cleveland, OH; B.D. Trapp, JHU, Balt, MD; R.A. Buchanan, Ark.State Univ., State Coll., AK; L. Pozzo-Miller, Roche Inst. Mol. Biol., Nutley, NJ

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Analytical Cell Biology,

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS: 1.15	PROFESSIONAL: 0.45	OTHER: 0.70

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This two-part project studies the organization and function of specialized membranes in neurons and glia. The first part aims to characterize calcium regulation during synaptic activity in parallel fiber/ Purkinje cell synapses of the cerebellar cortex and in synapses of CA3 hippocampal pyramidal cells. New frozen sectioning techniques, in combination with scanning transmission electron microscopy (STEM), have permitted studies of structure and calcium regulation in directly frozen preparations of a new kind of organotypic culture of hippocampus. Structural analysis in conjunction with electrophysiology recordings has demonstrated excellent organization of the CA3 mossy fiber synapses in these cultures. We have also successfully obtained cryosections from these synapses, and preliminary elemental analysis has provided for the first time data on the concentrations and distributions of total calcium in a hippocampal cultures with intact circuitry. In part two, the expression, transport and assembly of specialized myelin membrane proteins are studied. Confocal light microscopy had previously shown that Schwann cells depend on microtubules for intracellular transport and assembly of myelin-specific proteins. We have now used microtubule assembly/disassembly experiments to show that the P_0 protein, the myelin-associated glycoprotein (MAG), and laminin, which are targeted to discrete and different plasma membrane domains, are sorted into separate carrier vesicles as they exit the trans-Golgi network. Following sorting, certain carrier vesicles are transported along the myelin internode on microtubules, even though microtubules do not appear to selectively target these vesicles to specific membrane sites.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02836-04 LN

PERIOD COVERED

Octoberr 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural and Elemental Analysis of Macromolecular Assemblies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS *(if any)*

R.D. Leapman & S. Sun, BEIP, NCCR, NIH, Bethesda, MD; J.A. Hunt, Lehigh University, Bethlehem, PA.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Analytical Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.70	0.50	0.20

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to characterize the shape, molecular weight distribution and elemental composition of specific individual macromolecules and macromolecular assemblies. Such assemblies are critical to many cell functions, and their behavior in vitro reflects their function and regulation in intact cells. This project depends on a unique instrument - a low-temperature, high-resolution, field-emission scanning transmission electron microscope (STEM)-for molecular weight mapping and chemical analysis by parallel electron energy loss spectroscopy (EELS) of directly frozen thin films and ultrathin cryosections of directly frozen tissues. Dark-field molecular weight mapping of native neurofilaments (NFs) from squid axoplasm indicated that the mass per unit length of squid NFs was 38 = B15 kDa/nm, similar to values reported for other NF assemblies. Second-difference EELS spectra revealed that there are 30 P atoms within the 10-nm region of the NF, corresponding to complete phosphorylation of available KSP sites on the heavy subunit of these filaments. These results indicate that EELS is capable of detecting physiologically relevant differences in phosphorylation states at a useful spatial resolution of 10-20 nm. We have applied a new method-based on analyzing the valence electron region of a low-dose EELS map of frozen-hydrated sections to determine the distribution of water within mouse liver and within cerebellar cortex that had undergone trauma-induced cellular swelling. This method in combination with high-dose EELS spectrum imaging is expected to reveal trauma-related changes in water and calcium distributions at the subcellular level.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02834-04 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of the Excitation-Contraction Coupling Apparatus in Muscle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Bernhard E. Flucher, Ph.D.	Visiting Scientist	LN, NINDS
Others:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS
	Asher Shainberg, Ph.D.	Guest Researcher	LN, NINDS

COOPERATING UNITS (if any)

M.P. Daniels, LBG, NHLBI, NIH; J.A. Powell , Smith,Northampton, MA., C. Franzini-Armstrong, U. Penn. Philadelphia, PA.; K. Beam, Colorado State, Fort Collins, CO; J. Schmidt, SUNY, Stony Brook, NY.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Analytical Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS: 1.20	PROFESSIONAL: 1.10	OTHER: 0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to determine the molecular mechanisms involved in the assembly of the triad junction between T-tubules and sarcoplasmic reticulum (SR) during the development of excitation-contraction (E-C) coupling in skeletal muscle. Immunofluorescence studies of the distribution of the skeletal muscle dihydropyridine receptor (DHPR) (the putative voltage sensor in E-C coupling), the ryanodine receptor (RyR) (the calcium release channel of the sarcoplasmic reticulum) and triadin in developing normal muscle and dysgenic (mdg) myotubes in culture showed that a protein-protein interaction mediated by the DHPR plays a role in the normal organization of the triad proteins. The $\alpha 1$ subunit of the DHPR is essential for the normal targeting of the $\alpha 2$ subunit; it also facilitates the normal organization of the RyR and triadin although it is not absolutely required. *De novo* expression of the DHPR $\alpha 1$ subunit from normal nonmuscle nuclei fused with dysgenic myotubes restored normal functions and normal molecular organization of the E-C coupling membranes. Recordings of cytoplasmic free calcium with fluorescent indicators revealed that only action potential-induced calcium transients are eliminated in the dysgenic mutant. Calcium-induced calcium release events were essentially unaltered in the DHPR null mutant, suggesting that these events represent properties of the RyR independent from interactions with the DHPR and from its normal distribution in the junctional face of the terminal SR cisternae. A study of the role of calcium in transcriptional regulation in skeletal myotubes showed that depolarization-induced down-regulation of the acetylcholine receptor α subunit is mediated by calcium influx through the DHPR but not by calcium release from the SR. Thus, L-type calcium currents, depolarization- and calcium-induced calcium all release play different roles in the development and function of skeletal muscle.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01805-26 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Membrane Structure of Astrocytes

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Milton W. Brightman, Ph.D.	Section Chief	LN, NINDS
Others:	Elena Sanovich, Ph.D.	Special Volunteer	LN, NINDS

COOPERATING UNITS (*if any*)

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Brain Structural Plasticity

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.30	PROFESSIONAL: 0.30	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project is being held in abeyance until fiscal year 1995.

PHS 6040 (Rev 1-84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02086-21 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regeneration Specificity in Transplanted Neural Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David L. Simpson, Ph.D.	Special Expert	LN, NINDS
Others:	Milton W. Brightman, Ph.D.	Section Chief	LNC, NINDS

COOPERATING UNITS *(if any)*
Jung-Hwa Tao-Cheng, Ph.D., EM Facility, NINDS, NIH; Joseph Bressler, Ph.D., Kennedy Institute, Baltimore, MD

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Brain Structural Plasticity

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.30	PROFESSIONAL: 1.30	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work on how PC12 cells can be neuronally differentiated has taken two directions. (A) The use of doubly treated cells is a model system for synaptic vesicle properties. When PC12 cells are treated with both nerve growth factor (NGF) and ras-oncogene, the small 40-60 nm-wide vesicles in neurite varicosities more closely resemble such vesicles *in vivo.* The number of vesicles increases and, for the first time in a cell line, form clusters as they do *in vivo.* Aggregation enabled unequivocal immunocytochemical localization of synapsin I and synaptophysin to the vesicles and establish them as synaptic. By gel electrophoresis, the amounts of synapsin I levels rose in the treated PC12 cells while synaptophysin fell in NGF-treated cells. Other properties of synaptic vesicles can be probed with this system. (B) Identification of transcription patterns created by NGF and ras. Most, but not all, of the effects of NGF on PC12 cells can be mimicked by transduction with ras. It is still unknown as to which elements are critical for NGF differentiation and which genes are induced in the process. The technique of differential display of reverse transcribed mRNA from PC12 cells is being used to identify changes in mRNA expression. Eight transcripts were increased in NGF and ras cells and a ninth was abundantly expressed in ras but not in NGF treated cells. The pathway of neuronal differentiation by the two stimulations are closely parallel but not equivalent.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02869-03 LN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Influence of Leukocytes on Neural Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shigeru Naito, M.D.	Visiting Fellow	LN, NINDS
Others:	Lisa Chang, B.S.	Biologist	LN, NINDS
	Sayed Ahmed	Summer IRTA	LN, NINDS
	Milton W. Brightman, Ph.D.	Section Chief	LN, NINDS

COOPERATING UNITS *(if any)*

Lloyd Guth, M.D. Department of Biology College of William & Mary, Williamsburg, VA

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.90	PROFESSIONAL: 0.40	OTHER: 0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal is to determine whether axons of a damaged spinal cord can be induced to regenerate. As injections of exogenous, activated macrophages have not led to appreciable axonal regeneration across the cord lesion, attempts are now being made to inhibit some of the deleterious events such as cyclo-oxygenase generation by administering indomethacin and the monoamine oxidase inhibitor, deprenyl. The spinal cord of adult rats is crushed epidurally , at T-8 of rats, by compressing the cord between the blades of a fine forceps, separated by a 0.5 mm thick metal stop. Axons are identified by immunostaining for neurofilaments. As the pathological changes in damaged cords are variable, so are the number of intact axons. Immunostaining of GAP-43 at high dilution, is being continued to see if intact axons can be distinguished from regenerating ones in rats being treated currently. The cell types congregating at the lesion are being identified immunohistochemically to see if there is a predominant type that might be associated with the greatest number of regrowing axons.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02144-20 LN

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Blood-Brain Barrier

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shigeru Naito, M.D.	Visiting Fellow	LN, NINDS
Others:	Elena Sanovich, Ph.D.	Special Volunteer	LN, NINDS
	Lisa Chang, B.S.	Biologist	LN, NINDS
	Milton W. Brightman, Ph.D.	Section Chief	LN, NINDS

COOPERATING UNITS *(if any)*
Philip Friden, Ph.D., Alkermes, Inc., Boston, MA

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Brain Structural Plasticity

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.15	PROFESSIONAL: 2.00	OTHER: 1.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(A) The hypothesis that a blood vessel's structure is determined by the target tissue it supplies and not by the vessel's source, does not apply to mature tissue but rather, to fetal tissue. Instead of adult skeletal muscle autografts being exclusively vascularized by muscle type of continuous vessels (CV), grafts also contain fenestrated vessels (FV) of mature choroid plexus; FV are retained rather than being totally replaced by CV. The hypothesis does hold for fetal tissue up to a certain stage of development. E14 and E16 fetal muscles were grafted to the choroid plexus of adult hosts. In E14 grafts, ~80% of the muscle capillaries were CV, like those of brain or muscle. Very few of the CV were, however, from brain because there was no immunostaining of brain barrier antigen. In E16 grafts, ~70% of the vessels were FV. It is concluded that only target tissue need be fetal for the hypothesis to apply. Donor fetal tissue is being labeled with bromodeoxyuridine and ^{3}H-thymidine to identify vessel sources. (B) The mechanism of opening the blood-brain barrier (BBB) with RMP-7, a bradykinin analog, to small molecules such as sucrose and inulin (5 kD), is being examined by light and electron microscopy. Biotinylated dextran (3 kD) passes the barrier only very infrequently and in small amount whereas exudates of the smaller and highly cationic La^{3+} are much more consistent and escape by way of interendothelial clefts rather than vesicular transcytosis.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 00813-33 LNC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzymological Aspects of Neural Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	R. Wayne Albers, Ph.D.	Section Head	LNC, NINDS
Others:	Alexander Wheaton	Biologist Lab Technician	LNC, NINDS

COOPERATING UNITS *(if any)*
J.P. Froehlich, Ph.D., M.D., NIA, NIH, Baltimore, MD; K. Fendler, Max-Planck-Institut für Biophysik, Frankfurt, FRG; K. Taniguchi, Dept. Chem., Hokkaido Univ., Hokkaido, Japan

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Section on Enzyme Chemistry

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is comprised of research into the structure and functioning of ion transport systems. There are currently two active subprojects: (1) Transient kinetics: A collaborative study with Froehlich and Fendler.on the source of the transmembrane current that is generated by phosphorylation of the sodium pump has been completed. Collaboration with Froehlich on the source of the biphasic characteristics of phosphorylation and dephosphorylation is continuing. (2) Investigation of posttranslational modifications of the sodium pump. This project involves characterization of identified fragments of the sodium pump catalytic subunit by mass spectrometry.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02723-08 LNC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Peptides in the Adult and Developing Vertebrate Nervous Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harold Gainer, Ph.D.	Chief	LNC, NINDS
Others:	Susan Wray, Ph.D.	Research Cell Biologist	LNC, NINDS
	Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS
	Christopher Flores, Ph.D.	PRAT Fellow	LNC, NINDS
	Margi Goldstein, Ph.D.	Senior Staff Fellow	LNC, NINDS
	Diane Witt, Ph.D.	IRTA Fellow	LNC, NINDS

COOPERATING UNITS *(if any)*

Dr. M. Castel, Hebrew University, Israel; Dr. M. Morris, Wake-Forest University, Winston-Salem, NC; Dr. C. Collins, LAS, NINDS

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.45	3.2	0.25

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to study mechanisms underlying the developmental and homeostatic regulation of the gene expression, posttranslational modification, and secretion of neuropeptides with a focus on the magnocellular oxytocin and vasopressin neurons in the hypothalamus. Efforts are being made to develop *in vitro* (dissociated cell and slice explant cultures) and *in vivo* (transgenic mouse) model systems to study these issues. Preliminary results indicate that both types of model systems are close to being achieved. The approach is cell-biological and hence, the focus is on the activity and gene expression at the single cell level. For this purpose, immunocytochemical, *in situ* hybridization histochemical, and single cell mRNA techniques are utilized.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02724-08 LNC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Basic Mechanisms Using the Squid Nervous System Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Harold Gainer, Ph.D.	Chief	LNC, NINDS
Co-PI:	Harish C. Pant, Ph.D..	Research Chemist	LNC, NINDS
Others:	Shirley B. House, B.S.	Biologist	LNC, NINDS
	Philip Grant, Ph.D.	Special Volunteer	LNC, NINDS
	Hemin Chin, Ph.D.	Research Cell Biologist	LNC, NINDS
	Howard Jaffe	Special Expert	LNC, NINDS

COOPERATING UNITS (if any)

A. Giuditta, Ph.D., Institute of Biophysics, Naples, Italy; M. Tytell, Ph.D., Wake-Forest University, Durham, NC.

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology/Enzyme Chemistry

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.65	1.15	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our previous studies of protein synthesis in the squid giant axon system (Lasek et al., J Cell Biol 74:501-23, 1977; Gainer et al., J Cell Biol 74:524-30, 1977) revealed that *de novo* protein synthesis occurs in neuronal cell bodies but not from axoplasm from the giant axon. Recent studies have shown that the squid giant axon contains elements of the protein synthetic machinery, i.e., polysomes and mRNAs (Perrone et al., J Neurochem 49:698-704, 1987; Guiditta et al., J Neurochem Res 28:18-28, 1991) in addition to tRNA. These investigators suggested that protein synthesis can occur in the axonal compartment of the giant axon system. We have generated specific antibodies to squid neurofilament (NF) proteins to test this hypothesis. Since we have shown NF mRNA is present in axons (Way et al., PNAS 89:6963-7, 1992), we used these antibodies in biosynthesis/immunoprecipitation experiments using the squid giant axon to test the hypothesis of axonal protein synthesis. These experiments confirmed robust biosynthesis of NF proteins in squid stellate ganglia, but we failed to detect any NF protein biosyn-thesis in the giant axon.

We also examined the localization of NFs in adult tissues and during neural development using NF protein-specific antibodies. These studies showed that NFs were present in adult neural tissues, primarily in selected fibers, with giant axons showing the most robust expression. After the first neurons differentiated at stage 22, immunoblots showed NF60 and NF70 immunoreactive proteins at all stages. The NF 220 subunit, however, was not detected in immunoblots at any developmental stage. Phosphorylated NF 220 immunoreactivity, however, though absent in immunoblots was first seen in selected fibers of the stellate ganglia at stage 25, increasing thereafter, in all giant fibers until hatching (stage 30). The stellate ganglion is the first neural tissue to acquire a mature neurofilament complement (i.e., phosphorylated NF 220), shortly before the onset of jet-propelled escape behavior. The temporal pattern of expression of the NFs during development resembled that seen in vertebrates, i.e., the smaller NFs appeared before the larger subunits in most neural tissues. In the squid, the expression pattern seems to depend upon a post transcriptional regulation of a single gene rather than by transcriptional regulation of three independent genes as in vertebrates.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02725-08 LNC

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Phosphorylation and Regulation of Cytoskeleton in Neuronal Systems

P.I.:	Harish C. Pant, Ph.D.	Research Chemist	LNC, NINDS
Others:	Howard Jaffe, Ph.D.	Special Expert	LNC, NINDS
	William T. Link, Ph.D.	Research Chemist	LNC, NINDS
	Veeranna, Ph.D.	Visiting Fellow	LNC, NINDS
	Alexander Wheaton	Biologist Lab Technician	LNC, NINDS
	Niranjana Amin	Senior Staff Fellow	LNC, NINDS
	Kurudunje Shetty	Visiting Scientist	LNC, NINDS
	Megumi Takahashi	Special Volunteer	LNC, NINDS

COOPERATING UNITS *(if any)*

Dr. S. Beushausen, LN, NIH, NINDS.

LAB/BRANCH

Neurochemistry, BNP, DIR, NINDS

SECTION

Enzyme Chemistry

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.05	4.65	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying nervous system specific kinases and phosphatases to understand the role of neurofilament phosphorylation. Previously, we have shown that second messenger dependent and independent kinases can phosphorylate the neurofilament proteins (NFPs) *in vitro*; however, the kinases involved in phosphorylation *in vivo* are unknown. In order to determine the kinases involved in this phosphorylation, we have analyzed the endogenous phosphorylation sites in carboxy-terminal tail domain of high molecular weight neurofilament protein (NF-H), where most of the *in vivo* phosphorylation takes place. These studies showed that serines (s) in all three KSP motifs: KSPXK, KSPXXK and KSPXXXK are phosphorylated. Cyclin-dependent kinase, Cdk-5, from rat spinal cord or bovine brain, specifically phosphorylated KSPXK motifs. Purification of this kinase from rat spinal cord showed that it is associated with a protein of 67KDa (p67). Removal of this protein from the complex resulted in a considerable loss of the kinase activity which could be restored by adding back the purified or bacterially expressed p67. The complete amino acid sequence of p67 deduced from a number of cDNA clones from rat brain cDNA libraries appeared to be identical to Munc-18, a synaptic vesicle complex protein. The p67 transcript expression begins early in development and is restricted to the nervous tissue. Immunohistochemical staining with an amino-terminal peptide-specific antibody indicated that p67 is expressed exclusively in neurons. Localization in brain tissue and in cultured rat hippocampal neurons demonstrated that p67 is highly enriched in axons. We also investigated the expression of cdk-5, p67 and phosphorylated NF-H during development of the rat cerebellum from postnatal day 2 to adult using specific antibodies to these molecules. We could demonstrate that at all stages the epitopes for Cdk-5 and phosphorylated NF-H were colocalized in developing afferent fibers (mossy and climbing fibers) as well as basket cell fibers surrounding the Purkinje cells. Purkinje cell bodies, on the other hand, contained high levels of dephosphorylated NF-H and NF-M. These results are consistent with the hypothesis that cdk5 regulates the phosphorylation of some of the KSP motifs in neurofilament proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02757-07 LNC

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological Studies of Peptidergic Neurons and Peptide Receptors

P.I.:	Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS
Others:	Harold Gainer, Ph.D.	Laboratory Chief	LNC, NINDS
	Susan Wray, Ph.D.	Research Biologist	LNC, NINDS
	Susan Fueshko, Ph.D.	IRTA Fellow	LNC, NINDS
	Shirley House, B.S.	Biologist	LNC, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurochemistry, BNP, DIR, NINDS

SECTION
Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.35	PROFESSIONAL: 1.0	OTHER: 0.35

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electrophysiological studies of luteinizing hormone releasing hormone (LHRH) neurons in the hypothalamus were done by culturing explants of olfactory pit regions of embryonic mice (E-11.5) where LHRH neurons are abundant. LHRH neurons were maintained up to 3 weeks in these explant cultures and their electrical and synaptic properties were studied using patch-pipette, whole-cell current and voltage-clamp techniques. Cells were marked intracellularly with Lucifer yellow (LY) after recordings were made, and their phenotypes were subsequently identified immunocytochemically. Sixty-two cells were unequivocally identified as LHRH-immunoreactive. Morphologically, these cells were unipolar or bipolar, and resembled LHRH neurons found *in vivo*. Cultured LHRH-neurons had resting potentials of about -50 mV and exhibited tonic to partially phasic spontaneous discharges generated by both endogenous and synaptic mechanisms. Voltage-clamp analysis of the somatic membranes of the LHRH neurons revealed the following voltage-sensitive and insensitive ionic currents: at least two types of K currents - a transient current (I_A) and a delayed rectifier current (I_K), a tetrodotoxin (TTX)-sensitive Na current, and low threshold and high threshold Ca currents. Direct application of GABA-induced depolarizations at the resting membrane potential, due to Cl-conductance increases which were inhibited by picrotoxin or bicuculline. Spontaneous depolarizing synaptic potentials (in >10-day cultures), which exhibited the same reversal potentials as GABA-induced potentials, were also abolished by picrotoxin or bicuculline. These observations indicate the presence of functional $GABA_A$ synapses on these neurons. The cultured LHRH neurons were also depolarized by glutamate. GABAergic neurons which were multipolar, and possessed similar voltage-dependent ion channels and receptors as those of LHRH neurons, were also present in the cultures. Neurons of the nucleus of the diagonal band (DBN), supraoptic nucleus (SON) and paraventricular nucleus (PVN) of PN 5-10 rats were dissociated and cultured on the monolayer of the brain astrocytes for about 1 month. Following electrophysiological analyses by patch pipettes, the phenotypes of the recorded neurons were identified by double-labelling immunocytochemical methods. Both phasically and tonically firing spontaneous discharges were recorded from clearly visible neurons on the inverted microscope. Using these preparations, Ca-sensitive dyes Fura-2 (5K) or Ca green were introduced intracellularly and the dynamic states of intracellular Ca ions in these peptidergic neurons were analyzed by the Ca imaging/photometry set-up, which was coupled to the voltage-clamp system.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02820-05 LNC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning and Functional Analysis of Genes Active in Neurogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Ward F. Odenwald, Ph.D.	Senior Staff Fellow	LNC, NINDS
Others:	Ravi Kambadur, Ph.D.	Visiting Associate	LNC, NINDS
	Shang Ding Zhang, M.D.	Visiting Associate	LNC, NINDS
	Peter Vos, Ph.D.	IRTA Fellow	LNC, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology (Neurogenetics Unit)

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this program is to identify and functionally characterize neurogenic genes that are required for CNS development. Given the high degree of conservation observed in basic mechanisms utilized by all metazoans, our search for these genes was initiated in the fruit fly (*Drosophila melanogaster*) where developmental information is more accessible for study. Using classical genetic, molecular biology and transgenic techniques, we have continued to study the function of castor, a novel Zinc finger gene required for proper CNS neuroblast development and pollux, a novel cell-adhesion protein encoding gene also expressed in the developing CNS. We hypothesize that the castor protein functions as a DNA-binding transcription factor required for the regulation of genes involved in neuroblast development. Consistent with its proposed role, our recent studies have shown that the castor encodes a nuclear protein that complexes with DNA *in vivo*. To test our hypothesis, we are currently characterizing genomic DNA clones that contain *in vivo* castor protein DNA-binding sites to identify genes that may be regulated by castor. We have also pursued the characterization of cognate genes by first cloning castor and pollux cognates from other dipteran species. Information obtained from these comparisons will aid in our identifying mammalian cognates. Protein data bank searches have revealed that pollux is related to three human proteins. Recent studies on pollux have shown that it encodes a membrane associated protein that can serve as a ligand for RGD-binding integrins. Analysis of pollux mutant alleles revealed that it is required for proper trachea function. During our studies on pollux, we serendipitously observed high levels of male homosexual behavior in transgenic flies. Genetic and mutant analysis of these lines demonstrated that the behavior is triggered by the hyper or ectopic expression of the transgene vector's selectable eye marker, the mini-white gene. We have also continued our functional analysis of the murine homeobox gene A5. Ectopic expression of A5 in transgenic mice correlates with the apparent repression of a hepatocyte nuclear transcription factor (HNF-3b) in adult tissues. We are now assessing if *in utero* ectopic A5 expression modulates HNF-3b expression during development.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02824-04 LNC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development and Regulation of the Luteinizing Hormone Releasing Hormone System

PI:	Susan Wray, Ph.D.	Research Cell Biologist	LNC, NINDS
Others:	Sharon Key, B.S.	Biologist	LNC, NINDS
	Susan Fueshko, Ph.D.	IRTA Fellow	LNC, NINDS
	Jennifer Maurer, Ph.D.	Pratt Fellow	LNC, NINDS
	Kiyoshi Kusano, Ph.D	Visiting Scientist	LNC, NINDS
	Harold Gainer, Ph.D.	Chief	LNC, NINDS

COORPERATING UNITS (if any)

Dr. S. Radovick, Div. of Endocrin., Child's Hospital, Boston, Ma.; Dr. R. Weiner, Reprod. Endocrin. Ctr, UCSF, San Francisco, CA; Dr. B. Olson, NICHD, MD. Dr. M. Ottinger, Univ. of Maryland, MD.

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.05	PROFESSIONAL: 2.05	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Luteinizing hormone releasing hormone (LHRH) neurons are derived from the olfactory placode and migrate into the brain, where they become integral members of the hypothalamic-pituitary-gonadal axis. To study the migratory mechanism(s) involved in LHRH neuronal movement into the CNS, we use normal and transgenic animals, as well as olfactory explants. In addition, long-term organotypic slice cultures are used to study mechanisms underlying intrinsic and trans-synaptic regulation of LHRH gene expression, peptide synthesis and secretion in postnatal differentiated LHRH neurons.

Working on the hypothesis that LHRH neurons migrate on peripherin positive (+) olfactory axons from the olfactory pit to diencephalon, we found that: (1) LHRH neurons do not express peripherin mRNA; (2) LHRH neurons do not express N-CAM mRNA, although olfactory axons are N-CAM+; and (3) olfactory pit cells differentially express peripherin mRNA and N-CAM mRNA, suggesting distinct populations. In embryonic explants, we distinguished N-CAM+ and peripherin+ axons, and verified that LHRH neurons moved via peripherin+ but not N-CAM+ axons. Examination of voltage- and ligand-gated channels on embryonic LHRH neurons revealed membrane characteristics of highly differentiated neurons. In addition, we have generated embryonic explants from transgenic mice expressing luciferase in LHRH neurons. When given luciferin, a detectable signal is measureable in lysed cells from these explants.

We examined second messengers active in LHRH cells and oxytocin (OT) cells maintained in organotypic slice explants. Forskolin and/or phorbol 12-myristate 13-acetate (PMA) treatment significantly decreased LHRH mRNA levels at 4 hr. In contrast, forskolin treatment significantly increased OT mRNA levels by 8 hr. Using actinomycin D (a transcription inhibtor), we determined neuropeptide mRNA turnover rates: LHRH mRNA has a very fast turnover rate (~4 hr), while OT mRNA is much slower (~40 hr). We propose that second messengers act primarily to increase transcription of OT mRNA in OT cells, but decrease LHRH mRNA transcription and/or increase LHRH mRNA degradation in LHRH neurons.

Currently, we are determining: (1) cell surface glycoproteins expressed on LHRH neurons and/or the peripherin+ axons with which they associate; (2) the identity of cells expressing N-CAM vs those expressing peripherin in nasal regions; (3) whether LHRH neurons maintained in cultures release LHRH in a pulsatile manner; and (4) whether tagged-LHRH neurons can be visualized *in situ* to monitor movement in embryonic explants and/or determine the membrane properties of postnatal LHRH neurons in organotypic slices.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 NS 02828-04LNC

PERIOD COVERED
October 1, 1993 through Spetember 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Analysis of Calcium Channel Gene Expression in Mammalian Nervous System

P.I.:	Hemin R. Chin, Ph.D.	Research Cell Biologist	LNC, NINDS
Others:	Dong-Sun Kim, Ph.D.	Visiting Fellow	LNC, NINDS
	Oh-Joo Kwon, M.D., Ph.D.	Visiting Fellow	LNC, NINDS

COORPERATING UNITS *(if any)*
D.G. Puro, M.D., Ph.D. University of Michigan School of Medicine
C.A. Kozak, Ph.D. LMM, NIAID, NIH

LAB/BRANCH
Neurochemistry, BNP, DIR, NINDS

SECTION
Section on Molecular Neuroscience

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.9	PROFESSIONAL: 2.9	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects
  [ ] (a1) Minors
  [ ] (a2) Interviews
[ ] (b) Human tissues
[x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long-term goals of this project are to: (1) understand the molecular basis for cell type-specific expression of voltage-sensitive calcium channel (VSCC) genes; and (2) study the roles of VSCCs and synaptic vesicle-associated proteins in the process of secretion at peptidergic nerve terminals. Electrophysiologic and pharmacologic studies have classified voltage-sensitive Ca^{2+} channels (VSCC) into five major types: an emerging family of a low voltage-activated channels, and 4 classes of high voltage-activated channels (L-, N-, P-type, and the recently identified dihydropyridine-insensitive, ω-conotoxin-insensitive Q-type channels). Expression of these and other Ca^{2+} channel subtypes appears to be highly regulated both spatially and temporally during development of the central nervous system. To examine the molecular regulatory mechanisms underlying cell-specific expression of VSCC genes, we have chosen human N-type (α_{1B}) and L-type (α_{1D}) $\alpha 1$ subunit genes since expression of N-type channels is restricted to neurons particularly in nerve endings while L-type channels are present in many excitable cell types and also predominantly in neuronal perikarya. Although they are comprised of similar polypeptide subunits, these two high voltage-activated calcium channels are encoded by distinct genes located on separate chromosomes. During the past year we have carried out molecular analysis of the 5' flanking promoter regions of human L- and N-type VSCC genes to identify and characterize important regulatory elements that either enhance or repress expression of these genes in cell type- or tissue-specific manner. In addition, using subunit-specific antisera that we have prepared past year, we have purified rat brain N-type Ca^{2+} channels and identified polypeptide subunit components. The 160 KDa $\alpha_2\delta$ and 55 kDa β_3 subunit proteins were copurified with the ^{125}I-ω-CgTx-labeled α_1 subunit of N-type Ca^{2+} channels. The purified rat brain N-type Ca^{2+} channel fractions contained the vesicle docking proteins syntaxin and Munc-18 proteins, but not the synaptic vesicle proteins synapsin and synaptophysin. Thus, our results suggest that certain presynaptic membrane proteins can physically associate with the N-type Ca^{2+} channels, and this interaction may be an important regulatory mechanism for neurotransmitter release at the presynaptic terminals. In the coming year we plan to identify VSCC subtypes present in the peptidergic nerve terminals and characterize their interactions with synaptic vesicle-associated proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02874-02 LNC

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Studies of $GABA_A$ Receptor Expression During CNS Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Lawrence C Mahan, Ph.D.	Research Cell Biologist	LNC, NINDS
Others:	Peng-Xin Lin, M.D.	Visiting Associate	LNC, NINDS
	Peter M. Geiger	Biologist	LNC, NINDS

COOPERATING UNITS *(if any)*
A. Thierry, LTCB, NCI; M. Eiden, LCB, NIMH

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Molecular Neurosciences

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have employed *in situ* hybridization histochemistry (ISHH) studies to investigate the embryonic and early postnatal expression of subunit mRNAs of the $GABA_A$ receptor in the developing rat CNS. The results demonstrate the early (E14-E15) and restricted expression of specific ($\alpha_{2/3/5} > \alpha_{1/4} >>> \alpha_6$; $\beta_3 > \beta_2 >> \beta_1$; $\gamma_{2S} >> \gamma_1/\gamma_{2L}/\gamma_3$) subunit mRNAs in certain brain regions concurrent with or shortly after neurogenesis. Little data exist as to the nature of the developmental cues that direct these patterns of expression. In addition, while there appears to be compelling evidence for a developmental role for GABA and its receptor, there is very little fundamental information about the functions, either individually or in combination, of specific subunits in embryonic neurons. We have investigated two *in vitro* systems: pluripotent stem cells, that differentiate under controlled conditions into neurons and cultured embryonic neurons of defined developmental stage and anatomic location that express GABA synthetic capability and subunits of the $GABA_A$ receptor. One such *in vitro* model, murine P19 embryonic carcinoma stem cells, undergo sychronous differentiation upon exposure to retinoic acid to produce GABAergic neurons (~50-60%), glia (20-30%) and some fibroblast-like cells with 72 hr. We have used PCR to determine the temporal expression (0-30 DIV) of 13 subunit mRNAs and initial characterizations parallel ISHH studies *in vivo*. Moreover, between 3-10 days after RA-induced differentiation, P19 neurons exhibit GABA-mediated membrane depolarization and increases in intracellular Ca^{2+}. Additional studies are to be carried out on differentiating embryonic neurons isolated from olfactory bulb and cerebellum. We are currently developing single cell mRNA/PCR techniques to correlate subunit composition in embryonic neurons with electrophysiological and other functional characterizations of channel activation. Our long-term goal is to use molecular biological approaches in particular, antisense phosphorothioate oligodeoxynucleotides and the expression of antisense episomal vectors, to alter the expression of subunits of the $GABA_A$ receptor in embryonic and terminally differentiated neurons. To this end, we have constructed alternative expression vectors and delivery systems capable of sustained expression in neurons. We hope that these approaches may permit manipulation of the earliest expression of subunit genes of the $GABA_A$ receptor under conditions of more defined environmental and cellular interactions to further eludicate a role for the expression of the GABAergic system during CNS development.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS 02898-01 LNP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Molecular Physiology of CNS Development

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Roland Somogyi, Unit Chief, LNP, BNP, DIR, NINDS

Others: (LNP, BNP, DIR, NINDS):
Xiling Wen, Visiting Scientist
Wu Ma, Senior Staff Fellow
Cecilia Pazman, Visiting Fellow
JoAnn Castelli, Cooperative Student
Veronica Dunlap, Technician
Jeffery L. Barker, Chief

COOPERATING UNITS (*if any*)

NINDS, LNB (David L. Simpson); NCI, LCO (Zoltan Olah); Pharmacological Institute, University of Bern, Bern, Switzerland (Joerg Stucki)

LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

SECTION

Molecular Physiology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda MD, 20892

TOTAL STAFFYEARS: 4.31	PROFESSIONAL: 2.81	OTHER: 1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

In order to study the expression of genes underlying physiological development of the CNS, we have developed a quantitative PCR technique for the enumeration of mRNA molecules. Over the course of rat cervical spinal cord development we have measured the expression of the GAD (glutamic acid decarboxylase) gene family. GAD, GABA receptors and neurofilament mRNAs (as a neuronal marker) appeared in parallel, suggesting that GAD plays a role in neurogenesis. The precisely quantitated time course of GAD was described by a mathematical model based on feedback regulation of GAD on its own expression. This model provides a working hypothesis, making experimentally testable predictions. Furthermore, an alternatively spliced transcript of GAD67, EP10, was most strongly, almost restrictively expressed during development and is implicated in the early functioning of GAD in the transition from proliferation to differentiation. The parallel decline of EP10 and nestin (a neuroprogenitor marker) and number of proliferating cells are in accord with this conjecture.

In order to identify other developmentally important genes, we have conducted a quantitative PCR survey of genes associated with intra- and intercellular signaling in the cervical spinal cord. Genes coding for neurotransmitter synthesizing enzymes and receptors, peptide factors and their receptors, and genes associated with neurogenesis were differentially expressed during development. In addition, members of the IP3 (inositol trisphosphate) receptor family, playing a central role in regulating the second messenger, Ca^{2+}, were distinctly regulated during neurodevelopment on the level of mRNA expression. We have established a new method based on arbitrarily-primed PCR enabling the parallel identification of hundreds of randomly selected expressed genes. Using this technique, we have compared RNA samples from NGF-treated and naive PC12 cells and E12, E18 and adult cervical spinal cord, and have identified on the order of 30 to 50 differentially expressed genes. The amplified cDNA fragments have been isolated, sequenced and compared to known genes in the GenBank and EMBL databases. Several differentially expressed sequences matched with known genes: L1 line element (a retrotransposon), ribosomal protein S3A, cytochrome C oxidase and Zn/Cu-dependent superoxide dismutase. Some of the genes identified here have also been detected by subtractive hybridization of growth-factor treated cell cultures. The results confirm that the method faithfully detects differentially expressed genes and is suitable for the compilation of unbiased gene expression spectra.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02330-17 LNP

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Biological Studies of Developing CNS Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.L. Barker, Chief, LNP, BNP, DIR, NINDS.
Others (LNP, BNP, DIR, NINDS): A.E. Schaffner, Biologist
W. Ma, Senior Staff Fellow
T.N. Behar, Microbiologist
S.V. Smith, Biologist

COOPERATING UNITS *(if any)*
NINDS, LMVP (L. Hudson); SAIC, Fairfax, VA (J. Hickman); Naval Research Laboratories, Washington, DC (D. Stenger)

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.92	PROFESSIONAL: 2.0	OTHER: 1.92

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Flow cytometry, discontinuous-gradient cell isolation, dissociated cell culture, immunoblots, cell migration, immunochemistry and *in situ* hybridization methods are applied to embryonic/early postnatal rat CNS tissues to study the development, differentiation and cellular distribution of transmitter, transmitter-related enzymes and their corresponding receptors. During the past several years, we have focussed primarily on GABA, which is transiently expressed in a widespread manner during CNS development before it becomes relatively restricted to fast-transmitting synapses in the adult where it often functions in an inhibitory manner. In FY 94 we investigated the following: 1) transcripts encoding three GABA-synthesizing GAD enzymes and those encoding most GABA receptor subunit proteins have been detected by *in situ* in progressively more regions of the developing CNS; 2) they appear to be expressed at all levels of the embryonic neuraxis, even during the period of intense neuroblast proliferation; 3) several distinct patterns of GAD and GABA transcript co-expressions in CNS development are apparent: one almost exclusively in cells of the neuroepithelial proliferative zone, one in many, if not most differentiating cells during embryogenesis and one differentiating during the postnatal period; 4) it is clear that some transcripts are only transiently detected for variable periods while others persist, becoming restricted to subpopulations; 5) taken together, the data show that transcripts encoding GAD and GABA receptors are more abundant and widely distributed during embryogenesis than after differentiation is completed; 6) chemotropic effects of GABA on embryonic spinal cord and cortical cells have been discovered with postmitotic neurons migrating to fM, pM and μM concentrations, in an age- and/or region-dependent manner; 7) both chemotactic (gradient-dependent) and chemokinetic (gradient-independent) effects of GABA have been recorded, which can be mimicked by GABAmimetics active at $GABA_A$ and $GABA_B$ receptors suggesting novel GABA receptor structure-activity relations; 8) pertussis toxin inhibits GABA-induced chemokinesis, but not chemotaxis induced by NGF, implicating G protein-mediated signal transduction in these novel effects of GABA's; 9) staurosporine inhibits both effects of GABA on cortical dissociates, implicating protein kinase activity in the motility and migration signals. 10) 10^{-18}-10^{-8} M GABA transiently increases cytoplasmic free Ca^{2+} in postmitotic progenitors and neuroblasts during the embryonic period of cortical development in an all-or-none manner; 11) this effect involves discharge of Ca_i^{2+} from an intracellular store that is sensitive to ryanodine; 12) 10^{-7}-10^{-5} M GABA depolarizes progressively more embryonic cortical cells in a Cl^--dependent manner and elevates Ca^{2+} by promoting its entry.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02019-22 LNP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Properties Developing on CNS Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.L. Barker, Chief, LNP, BNP, DIR, NINDS

Others (LNP, BNP, DIR, NINDS): A.E. Schaffner, Biologist
J Vautrin, Visiting Scientist
J-M. Mienville, Visiting Fellow
Q.Y. Liu, Visiting Fellow
R. Serafini, Visiting Fellow
Y.X Li, Visiting Fellow
H. Xian, IRTA Fellow

COOPERATING UNITS *(if any)*

ICS, NINDS (G.D. Lange)

LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 7.1	PROFESSIONAL:. 7.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [X] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electrophysiological and digital video microscopic techniques are used primarily to elucidate the development, differentiation and cellular distribution of physiological properties expressed by embryonic and postnatal mammalian CNS neurons. Electrical studies involve direct, continuous high-fidelity amplification of ion fluxes generated either in single cells or patches or in pairs of cells in culture. Optical recordings include indirect measurements of membrane potential or cytoplasmic Ca^{2+} (Ca_c^{2+}) in small populations (50-100) of cultured cells. Principal findings include: 1) regenerative Na_o^+-dependent action potentials and underlying voltage-dependent currents are expressed early in embryonic telencephalic and spinal neurons, when most of the cells are still actively proliferating; 2) electrical and dye coupling among cortical cells recorded *in situ* disappears during embryogenesis; 3) undifferentiated, large-conductance K^+ channels appear in the proliferating period with heterogeneous properties including unstable openings, variable voltage-dependence, Ca^{2+} sensitivity and kinetics; 4) K^+ channel properties differentiate during the rest of the embryonic period; 5) micromolar GABA activates Cl^- channels with heterogenous properties in spinal cord cells beginning during the proliferative period; 6) activation of Cl^- channels depolarizes virtually all embryonic neurons acutely recovered from spinal and supraspinal regions; 7) routine culture methods show that the depolarizing effects of GABA disappear progressively over a period of several weeks; 8) this transformation in polarity of GABA's Cl^--dependent voltage signal can be hastened by co-culturing cells on astrocytes or by using routine methods combined with medium conditioned by astrocytes; 9) initially GABA is released from embryonic neurons in a continuous, tonic manner capable of polarizing cells near E_{Cl} before it is discharged in transient pulses that generate synaptic-like events; 10) cultured embryonic hippocampal neurons secrete and receive GABA in a new form of fast communication ("cismission," same-sided signalling); 11) "cismitting" GABAergic neurons discharge GABA in transient pulses at negative potentials and in a continuous mode at depolarized potentials; 12) exogenous GABA sticks to the surface of embryonic neurons long after the application is over, as if the exposed surface promotes an unstirred equilibrium of GABA that randomly activates a perseverating Cl^- signal; 13) kinetic components of open Cl^- channels activated by GABA shorten during development in cells dissociated from spinal and supraspinal regions, as GABA becomes inhibitory in function.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01659-26 LNP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synaptic Contacts of Retinal Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. Lasansky, Unit Chief, LNP, BNP, DIR, NINDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The amplitude of the light-evoked inward current recorded from depolarizing retinal bipolar cells may be more than doubled by a mixture of 20 µM CNQX and AP-7. These glutamate antagonists also induce a steady outward current, reduce the baseline noise, and decrease the amplitude of the rod-driven inward current that persists after the end of a bright stimulus. Because the latter observations indicate that the effect of CNQX and AP-7 is more complex than simply suppression of the chloride-dependent input, the increase in amplitude of the light-evoked inward current cannot be used to estimate the intracellular chloride concentration.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02631-11 LNP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function in Retinal Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ralph Nelson, Unit Chief, LNP, NINDS
Others: Michael A. Freed, Staff Fellow, LNP, NINDS

COOPERATING UNITS *(if any)*

Physiology, University of Vienna, Austria (Renate Pflug)
Physiology, University of Utah School of Medicine, Salt Lake City (Helga Kolb)
Psychology, Queens College, City University of New York (Thomas Frumkes)
Anatomy, University of Pennsylvania, Philadelphia (Peter Sterling, Robert G. Smith)

LAB/BRANCH

Laboratory of Neurophysiology, DIR, NINDS

SECTION

Neural Circuitry Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda Maryland 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neural organization and neural interactions in mammalian retinas are investigated using intracellular electrophysiology, electron microscopy, and pharmacology. A flat mount, superfused retina preparation has been devised for the cat. In this preparation, electrodes are aimed under visual control at retinal neurons, and penetration is verified by immediate staining of the impaled cell. Intracellular recordings from retinal ganglion cells with 50 mV resting potentials, 40 mV action potentials, and 10 mV light-induced generator potentials could be maintained in excess of one hour. Space constants of horizontal cells (HC's) in rabbit and cat retinas were calculated from the amplitude of responses to a 200 μm-wide light bar presented at different positions across the receptive field. Dopaminergic drugs (35-750 μM) were administered through the perfusion media. Apomorphine consistently increased rabbit HC space constants (20-150%). Five other ligands including the D_1 specific agonist SKF38393, the D_1 antagonist SCH23390, the D_2 agonist quinpirole, the D_2 antagonist sulpiride, and dopamine itself induced only small (<25%), inconsistent changes in space constant. Apomorphine effects were evident in rabbit, but not cat. Dopaminergic ligands affect HC space constants, but may not follow classic patterns of D_1-D_2 agonism and antagonism.

ON-OFF amacrine cells in cat retina were studied electrophysiologically and stained with HRP for morphological and ultrastructural investigation. Stained cells were of three types: A19, A20 and A22. The types varied in level of branching in the inner plexiform layer, and in synaptic connectivity. Arborizations were monostratified and consisted of two zones: a central zone of dendritic branching, and a more distal zone of multiple axon-like processes. Gaussian receptive field radii (566 ± 100 μm) were larger than central dendritic zone diameters, reflecting signals from an area covered by the long axon-like processes. Predominantly rod signals were seen at ON, whereas evenly mixed rod and cone signals were seen at OFF. Reversal potentials for both ON and OFF components were 25-35 mV, associated with conductance increases from 0.1-0.4 nS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02767-07 LNP

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Image Processing and Analysis of Cellular Structures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: T. G. Smith, Jr., Unit Chief, LNP, BNP, DIR, NINDS
Others: (LNP): Anne E. Schaffner, Biologist
T. N. Behar, Technician

COOPERATING UNITS *(if any)*

NINDS, IACS (G. D. Lange, W. H. Sheriff, Jr.); NINDS, LNLC (W. B. Marks); NICHD, LDB (E. A. Neale, L. M. Bowers); NCRR, BEIP (Seth R. Goldstein, Michael Unser, Michael Vhrel); Leipzig University, Germany (Andreas Reichenbach, Kurt Brauer); Wurzburg University, Germany (Dieter Senitz); AO Systems Design, Laurel MD (Thomas Hubin).

LAB/BRANCH
Laboratory of Neurophysiology

SECTION
Unit on Sensory Physiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.18	PROFESSIONAL: 1.10	OTHER: 0.08

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued to employ the concepts of Mandelbrot's fractal geometry to the quantitative studies of central nervous system neurons, and other cell types grown in tissue culture or from whole animals. We do this by employing image processing techniques to measure the fractal dimension (D), which is a measure of the complexity of the structure under investigation. In particular, the D relates to the degree of branching (e.g., of dendrites), the ruggedness of borders and the degree of space-filling of the object of interest. We have undertaken, in separate studies, how the fractal dimension changes during the differentiation and growth of glial cells from different sources (optic nerve and brain) and neurons in tissue culture. We have found that both optic nerve and brain-derived oligodendrocytes differentiate faster and to a greater extent than do both types of astrocytes and that nerve-derived glia also differentiate faster and to a greater extent than do brain-derived glia. Interestingly, the rates of differentiation, as measured by D, can be described by a single time constant. The work on cultured spinal neurons shows that the cells can be classified into four groups on the basis of the number of their primary dendrites and that they differentiate in a similarly simple fashion, with each of the four groups having distinctive final values and time constants. We have proposed that D is a useful, quantitative measure of morphological differentiation. We examined the D's of Purkinje cerebellar cells from nine vertebrate species, ranging from birds through marsupials and mammals, including man. This indicates a phylogenetic constancy of Purkinje cell morphological complexity going back at least as far as birds in the evolutionary tree. We have begun studies of the development of the internal and surface structures of cultured rat hippocampal neurons with fluorescence and confocal microscopy in order to localize the position of GABA and glutamate boutons. We find that GABA boutons are located almost exclusively on somata and proximal dendrites, while glutamate boutons are mainly on peripheral dendrites but occasionally on proximal dendrites and less so on somata. We continue in our efforts to improve the performance of our confocal microscope with no moving parts by changes in design and components.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02608-11 LNP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Organelle Transport of Ion Channels in Excitable Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John R. Clay, Unit Chief, ICB, LNP, BNP, DIR, NINDS

COOPERATING UNITS *(if any)*

A. Kuzerian (Marine Biol. Lab., Woods Hole, MA); A. Shrier (McGill Univ., Montreal, Canada); J. Trimmer (SUNY at Stony Brook, NY); K. Pfister (Univ. of VA Health Sci.Ctr., Charlottesville, VA; S. Brady (Univ.of Texas Southwest Medical Center, Dallas, TX); H. Pant (LNC)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Unit on Ion Channel Biophysics

INSTITUTE AND LOCATION

NIH, NINDS,Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns the transport of ion channels in nerve axoplasm by intracellular organelles. Previous work in this project has demonstrated to distinct populations of organelles isolated from the axoplasm of squid giant axons; a putative anterograde organelle population having diameters in the 40-60 nm range, and a putative retrograde population having diameters in the 100-150 nm range. During the past year these two populations have been analyzed with electrophysiological and biochemical procedures. One key finding has been that immunoblots of the anterograde organelles display cross-reactivity to an antibody against kinesin, a 120 kD ATPase which translocates these organelles along microtubules in the nerve. No cross-reactivity to kinesin was observed in the putative retrograde organelles even though the antibody has been shown with differential interference contrast light microscopy to block transport of organelles in both the anterograde and retrograde directions. Preliminary work has also been carried out with antibodies raised against various portions of the C-terminal region of the rat brain potassium channel, Kv 2.1. One particular antibody shows specific crossreactivity in immunoblots to the retrograde organelles. However, the antibody does not block potassium current, I_K, in axons using the standard axial wire voltage-clamp technique, whereas other antibodies which do block I_K in the axon do not appear to exhibit cross-reactivity with either population of organelles in immunoblots. These results suggest that the I_K channel has biochemical differences depending upon its location, be it either in anterograde or retrograde organelles, or in the axolemma itself.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02034-22
LVMP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Oligodendrocyte Lineage of Rodent and Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Dubois-Dalcq, M.D.	Chief	LVMP,NINDS
Others:	R. Voskuhl, M.D.	Comm. Officer	LVMP,NINDS
	L. Milward, Ph.D.	IRTA	LVMP,NINDS
	R. Rusten	Biol. Lab. Techn.	LVMP,NINDS
	P. Paras	Biologist	LVMP,NINDS
	W. Farris	HHMI Student	LVMP,NINDS

COOPERATING UNITS *(if any)*

Dr. I. Duncan, Univ. of Wisconsin; Dr. C. Kufta, Neurosurgery Branch, NINDS; Dr. M. O'Connor, University of Pennsylvania, Philadelphia, PA; Lynn Hudson and Jim Kim, LVMP; H. McFarland, NIB,NINDS.

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Neural and Molecular Biology

INSTITUTE AND LOCATION

NNINDS, , NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.75	3.25	1.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myelin-forming cells enwrap axons to allow fast conduction along major nerve tracts. In multiple sclerosis (MS) and some CNS viral diseases, damage to myelin-forming cells result in important neurological dysfunction. Our studies of the human oligodendrocyte lineage demonstrate that the human myelinated brain contains a discrete subpopulation of glial cells expressing two oligodendrocyte-specific and developmentally regulated genes, the PDGF receptor alpha and the myelin transcription factor 1 (MTF1). Precursors of oligodendrocytes are also identified in cultures of adult human white matter. bFGF induces human oligodendrocytes to rapidly regenerate their processes and to dedifferentiate without going through mitosis. This suggests that phenotypic plasticity rather than mitogenic potential may account for the regeneration of myelin-forming cells in the adult human brain. We are therefore working toward the establishment of a human oligodendrocyte cell line by transferring growth factor receptor genes with the hope of obtaining oligodendrocyte mitosis in response to known growth factors. An oligodendrocyte growth factor dependent cell line has indeed been successfully established in the rat and shown to be able to myelinate myelin-deficient rats after grafting in the spinal cord. Moreover, such cells, transfected with a marker gene, were shown to migrate up to 12 mm along the dorsal column and integrate well into the white matter. Thus, rat oligodendrocytes progenitors can maintain their ability to migrate and myelinate in vivo after multiple passages. This suggest that such cell lines might be useful to correct genetic defects of myelination in mice and men.

Another focus of our research is on the mechanisms of remyelination and recurrent demyelination in rodent and men. During remyelination, there is an increase in myelin basic protein (MBP) transcripts that are characteristic of the premyelinating stage. Importantly, an immune response to specific peptide sequences encoded by these transcripts have been correlated with disease activity in familial forms of MS. We are therefore examining in two different animal models whether the reemergence of these MBP isoforms triggers a T lymphocyte response specific for these proteins and can be correlated with disease perpetuation.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 NS 02528-13 LVMP

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Control of Gene Expression in the Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Lynn D. Hudson, Ph.D.	Section Chief	LVMP, NINDS
Others:	Jin G. Kim, Ph.D.	Sr. Staff Fellow	LVMP, NINDS
	Claudi Wiese, Ph.D.	Research Volunteer	LVMP, NINDS
	Mukul Ranjan, Ph.D.	IRTA	LVMP, NINDS
	Arthur Warrington, Ph.D.	Research Volunteer	LVMP, NINDS
	Jodi Berndt, B.S.	Microbiologist	LVMP,NINDS

COOPERATING UNITS *(if any)*
H. Arnheiter, Section of Viral Pathogenesis, LVMP; H. DeF. Webster, LENP, NINDS; R. Armstrong, Dept. Anatomy, USUHS, V. Nikodem, Clin. Endocrinology Branch, NIDDK.

LAB/BRANCH
Laboratory of Viral and Molecular Pathogenesis

SECTION
Section of Developmental Genetics

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.7	PROFESSIONAL: 4.7	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms that dictate the final program of gene expression in a fully differentiated cell can be revealed by starting at either end of the regulatory cascade. To examine the series of controls operating on cells of the oligodendrocyte lineage, we have cloned a number of putative transcription factors that recognize one of the final targets of regulation in myelinating glial cells, proteolipid protein (PLP). One such factor named MyTI (myelin transcription factor I) is a novel member of the zinc finger superfamily. Several observations suggest that MyTI may be instrumental in early stages of oligodendrocyte development and myelin production. MyTI message is more highly expressed in oligodendrocyte progenitors than in differentiated oligodendrocytes. In progenitors, MyTI immunoreactivity appears as speckles within the nucleus, suggestive of an association of MyTI with spatially segregated functional domains. MyTI has two clusters of DNA-binding domains, one of which binds to a consensus site represented several times in the PLP promoter and also found in other myelin genes. MyTI may represent an emerging class of regulatory proteins with a combination of features that predicts a role in coordinating the expression of a set of genes. Characteristic features shared by such proteins would include a structure of multiple DNA-binding domains which are each stabilized by a divalent cation, initial expression that markedly precedes the target gene, and localization within discrete macromolecular compartments of the nucleus. The isolation of clones encoding transcriptional regulatory proteins permits a search for the growth factors and other cytokines that are critical to the initiation and maintenance of myelin gene transcription during development and regeneration. We have evidence for the molecular mechanism by which thyroid hormone affects the transcription of the PLP gene and thereby has an impact on myelination. A site for the thyroid hormone receptor (THR) was identified within the PLP gene and shown to be essential for hormonal responsiveness. A prerequisite for THR activation of this site is the dimerization of THR with another member of the nuclear hormone receptor family, PPAR, which is a regulator of lipid metabolism. By employing a family of transcription factors whose heterodimerization specificity determines the target specificity and ultimately allows the coupling of divergent signaling pathways, an oligodendrocyte may balance the requirements for myelin protein synthesis with those of myelin lipid synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02742-08 LVMP

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Viral Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Meier, Ph.D.	Sr. Staff Fellow	LVMP, NINDS
Others:	H. Arnheiter, M.D.	Visiting Scientist	LVMP, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Viral and Molecular Pathogenesis

SECTION
Viral Pathogenesis Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new family of large GTPases (M_r = 70,000 to 100,000) contains proteins which are structurally conserved in their amino-terminal halves but divergent in their biological activities. The alpha/beta interferon-induced Mx proteins of vertebrate species confer a high degree of resistance to specific RNA viruses. The constitutively expressed dynamin protein of *Drosophila* and vertebrate species plays a role in the early events of endocytosis. The yeast VPS1/SPO15 protein is required for exocytosis and spindle pole body separation, and the yeast MGM1 protein is essential for maintenance of mitochondrial DNA. The goal of our studies is to characterize more precisely the functions of the vertebrate members of this family.

In order to determine the cellular function of Mx proteins, we have started to identify cellular proteins that interact with Mx proteins and, therefore, may comprise their downstream or upstream targets.

To determine the biological function of dynamin we have initiated a project to eliminate its expression in mice by homologous recombination. We have isolated and characterized genomic DNA clones corresponding to the amino-terminal portion of the dynamin gene. We have generated and electroporated a targeting construct into embryonic stem (ES) cells and have isolated an ES cell clone with a mutated dynamin gene. Injection of this clone into blastocysts yielded four highly chimeric mice, three of which transmitted the agouti coat color marker to the offspring. Mice homozygous for the mutated dynamin gene will be analyzed for phenotypic manifestations and may serve as a model for neurologic disorders caused by defects in the endocytic pathway.

PHS 6040. 1

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02789-06 LVMP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotropism of Human Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	M. Dubois-Dalcq, M. D.	Chief, LVMP	LVMP, NINDS
Others:	S. Wilt, Ph.D.	IRTA Fellow	LVMP, NINDS
	K. Nagasato, M.D., Ph.D.	Special Volunteer	LVMP, NINDS
	J. M. Zhou	Visit. Associate Techn.	LVMP, NINDS
	R. Rusten	Biol. Lab. Techn.	LVMP, NINDS

COOPERATING UNITS (if any)

Dr. F. Chiodi, Karolinska Institute, Stockholm, Sweden; Dr. M. O'Connor, Univ. of Pennsylvania, Phil., PA and Drs. D. Griffin and S. Wesselingh, Johns Hopkins Medical School, Baltimore, MD.

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Neural and Molecular Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 2.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HIV-1 can infect the central nervous system (CNS) causing the AIDS psychomotor complex including the HIV-1-associated leukoencephalopathy and myelopathy

We have investigated the role of tumor necrosis factor (TNF) alpha in HIV-1 encephalopathy using purified microglial cultures derived from adult human brain. Such cells are activated and express TNF alpha, just as they do in the brain tissue of patients with AIDS psychomotor complex. When infected with HIV-1 in the continuous presence of TNF alpha antibody, HIV-1 expression and virus growth in microglial cells are strongly inhibited for over a week, suggesting that TNF alpha naturally produced in this *in vitro* system may enhance HIV-1 replication. Moreover, microglial cell-derived TNF alpha are toxic for oligodendrocytes, the CNS myelin-forming cells and may cause the demyelination observed in the HIV-1 leucoencephalopathy. To investigate this possibility, we have developed an *in vitro* assay in which TNF alpha induced-cell death of purified rat and/or human oligodendrocytes can be accurately measured.

Using this assay, we found that recombinant human TNF alpha as well as TNF-alpha derived from activated human microglia is toxic for 25-30% of rat and human oligodendrocytes *in vitro*. Moreover such toxicity can be induced by coculturing human microglia with human oligodendrocytes and neutralized in great part by TNF alpha inhibitors such as pentoxifylline (PTX), anti-TNF alpha antibodies and soluble TNF alpha receptors. As PTX is a potent inhibitor of both HIV-1 replication and TNF-alpha synthesis and is presently used in phase 1 trial in AIDS patients, it is possible that this drug may delay the onset of the AIDS psychomotor complex. Our ongoing studies aimed to determine (1) whether TNF-alpha can be cytotoxic for oligodendrocytes *in vivo*; and (2) the molecular basis of this cytotoxicity.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02790-06 LVMP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Insertional Mutations in Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Heinz Arnheiter, M.D.	Visiting Scientist	LVMP, NINDS
Others:	Colin A. Hodgkinson, Ph.D.	Visiting Fellow	LVMP, NINDS
	Atsuo Nakayama, M.D., Ph.D.	Visiting Fellow	LVMP, NINDS
	Karin Opdecamp, Ph.D.	Visiting Fellow	LVMP, NINDS
	Cathy Chen	HHMI-NIH Med. Res. Scholar	LVMP, NINDS
	Ellen Meier, Ph.D.	Senior Staff Fellow	LVMP, NINDS
	Susan Skuntz	Biologist	LMP, NINDS

COOPERATING UNITS *(if any)*

N. Jenkins, Ph.D., N. Copeland, Ph.D., E. Steingrimsson, Ph.D., ABL Bas. Res. Prog., NCI-CRDC Frederick; M. Tachibana, M.D., LMO, NIDCD; R. Balling, Ph.D., E Hustert, Ph.D., Max Planck Inst., Germany

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 6.0	PROFESSIONAL: 5.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In mice, mutations at the microphthalmia (mi) locus on chromosome 6 may lead to abnormalities that include loss of coat pigmentation, hearing impairment, microphthalmia, and osteopetrosis. These abnormalities result from deficiencies in osteoclasts, retinal pigment cells, and neural crest-derived melanocytes of the skin and inner ear. Using a transgenic insertion at mi, we have recently isolated a gene that resides at this locus and encodes a novel member of the basic-helix-loop-helix-zipper class of transcription factors. In all mi alleles analyzed to date, this gene is mutated; in many of them, the nature of the mutation helps explain the mode of inheritance and the interactions between mi alleles that have been observed previously in compound heterozygotes. A detailed analysis of the expression of this gene suggests that mi plays an important role early in melanocyte development in eye, inner ear, and skin. To understand more precisely how mi exerts its pleiotropic effect on different cell populations, we have established culture systems in which cell proliferation and differentiation can be studied in conjunction with analysis of expression of mi and other genes. Mutations at mi may serve as models for human Waardenburg syndrome type II, a hereditary syndrome characterized by varying degrees of hearing impairment and pigment alterations. The recent isolation and chromosomal mapping of the human counterpart of mouse mi, MITF, may help to clarify the molecular basis of this disorder and possibly other forms of syndromic hearing loss.

Another line of transgenic mice contains approximately 15 transgene copies integrated into an intron of the mouse mox1 gene that encodes a mesodermal homeodomain protein. Mice homozygous for this transgene integration lack expression of mox1 and show a phenotype characterized by atlanto-baso-occipital fusions, hemivertebrae, and short, kinky tails, but they are fertile and have a normal lifespan. Crosses with other mice with similar phenotypes but mutations in different genes, along with a detailed study of the expression of mox1 and other genes, will help to place mox1 into a genetic hierarchy of factors involved in the development of the vertebra and skull. Mox1 is localized on distal chromosome 11 near Tail short (Ts), a locus whose mutations are also associated with short, kinky tails. Whether Ts and mox1 are identical is currently being tested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02830-04 LVMP

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of HIV Transcription In Vitro and In Vivo

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. E. Verdin, M.D. Senior Staff Fellow LVMP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated May 29, 1993 when Dr. Verdin departed NIH.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02652-10 BFSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Collaboration and Consultation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, NINDS
	James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section	BFSB, NINDS
Others:	Paul S. Albert, Ph.D.	Mathematical Statistician	BFSB, NINDS
	Dallas Anderson, Ph.D.	Mathematical Statistician	BFSB, NINDS
	Gregory Campbell, Ph.D.	Chief, Analytical Biometrics Section	BFSB, NINDS
	Sherrie E. Emoto, Ph.D.	Mathematical Statistician	BFSB, NINDS
	Lisa McShane, Ph.D.	Mathematical Statistician	BFSB, NINDS
	Martin Kulldorff, Ph.D.	Guest Researcher	BFSB, NINDS
	Nicholas Lange, Ph.D.	Special Expert	BFSB, NINDS

COOPERATING UNITS (if any)

Bombay Hospital, India (Dr. N. Bharucha); Peking Union Medical College, PRC (Dr. Z. Zhang); NIMH (Dr. Norman Rosenthal); Institute for Stroke Research and Prevention, Austria (Dr. M. Brainin); Harvard Univ. Boston, MA (Dr Q Registein); Pharmacy, Clinical Center, NIH (Dr. K. Calis)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Office of the Chief, Mathematical Statistics Section, Analytical Biometrics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.33	PROFESSIONAL: 3.38	OTHER: 1.95

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project encompasses a wide scope of statistical collaboration and consultation with laboratories and branches within the Division of Intramural Research (DIR), and with other neuroscience units outside NIH. Particular consideration is given to statistical planning and design of experiments, statistical analysis of data, and statistical inference. Examples of current studies include: clinical trial of bioequivalence of α-mannose-terminated glucocerebrosidase from natural and recombinant source, clinical course and long-term outcome of patients treated with $\text{Ceredase}_{\text{TM}}$ in Gaucher's disease, evaluation of skeletal responses of patients with Gaucher's disease treated with $\text{Ceredase}_{\text{TM}}$,longitudinal study of the clinical course of Fabry's disease (DMNB); clinical trials of felbamate for the treatment of intractable complex partial seizures, measurement of the effect of time from last seizure and seizure type on metabolic change as measured by PET, study of epilepsy progression to general tonic-clonic seizures (ERB); clinical trial of the effect of cyclosporine on lesion development in relapsing-remitting MS, modeling lesion recurrence in relapsing-remitting MS, clinical trial of DSG on lesion development in relapsing-remitting MS, monitoring MRI T2 weighted imaging in relapsing-remitting MS; determination of the role of B7:CD 28/CTLA-4 interaction in chronic relapsing EAE; examining the effect of accumulation of disease white matter in patients with relapsing-remitting MS (NIB); prevalence study of neurologic diseases in the Navajo tribe (ERB); study of abnormal facilitation response to transcranial magnetic stimulation in PD patients, identification of deficits associated with "over use" syndrome in pianists, three clinical trials of IV/IG in neuromuscular disorders, follow-up study of twins discordant for paralytic polio and subsequent post-polio syndrome; clinical trial of amantadine for the treatment of post-polio fatigue (MNB); evaluation of neuronal sprouting and behavioral recovery in hemiparkinsonian rats after amnion cell transplantation (SNB); validation study of consultations provided by U.S. drug information centers; incidence study of nervous system tumors in Israel; incidence study of motor neuron disease on Guam (NEB); development of Markov models for rapidly cycling bipolar disorder, examination of the relationship between bright light exposure and hot flashes in menopausal women (NIMH); studies of silent stroke risk factors and their implication for survival of a subsequent stroke,and risk of seizures following stroke.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02490-14 BFSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Research in Statistics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section	BFSB, DIR
Co-PI:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR
Others:	Paul S. Albert, Ph.D.	Mathematical Statistician	BFSB, DIR
	Dallas W. Anderson, Ph.D.	Mathematical Statistician	BFSB, DIR
	Gregory Campbell, Ph.D.	Chief, Analytical Biometrics Section	BFSB, DIR
	Sherrie E. Emoto, Ph.D.	Mathematical Statistician	BFSB, DIR
	Lisa M. McShane, Ph.D.	Mathematical Statistician	BFSB, DIR
	Martin Kulldorf, Ph.D.	Guest Researcher	BFSB, DIR
	Nicholas Lange, Ph.D.	Special Expert	BFSB, DIR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.42	PROFESSIONAL: 2.42	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project addresses statistical problems generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics and other members of the Branch. Papers have been submitted, are in review or were published in FY 1994 on the following statistical subjects: evaluation of proxy respondents and auxiliary information as adjustments for nonresponse or attrition in disease surveys; modeling time series for count data from a relapsing-remitting disease; modeling seasonal change in time series regression relationships; development of both discrete and continuous time Markov models for longitudinal categorical data which allow for random and different processes across subjects; derivation of statistical methods for the detection of and tests of differences among spatial disease clusters. Other work in progress includes: methods to improve coverage in surveys; estimation of time-to-event data with interval censoring; site selection for epidemiologic surveys; analysis of response surface data with spatial and temporal components; modeling of response surfaces with spatially correlated errors; application of splines to estimate model parameters of multiple correlated response surfaces; modeling effect changes of covariates in the presence of spatial correlation; analysis of bioequivalence trials with multiple, nonlinear responses to treatment; combining information from negatively correlated nonlinear regressions; development of a generalized estimating equation approach for the analysis of spatially dependent binary data; application of bootstrap methods to longitudinal natural history data for the design and analysis of therapeutic trials for relapsing-remitting disease; use of variance component methods to assess the precision of biochemical measurements; using a Markov chain model to study three state disease processes; and sampling strategies for spatial point processes with multiple types of clustering.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02879-02 BFSB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistics and Neuroimaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Gregory Campbell, Ph.D.	Chief, Analyt. Biomet. Sect.	BFSB, DIR
Others:	Nicholas Lange, Ph.D.	Special Expert	BFSB, DIR
	Alan Polis	Computer Systems Analyst	BFSB, DIR

COOPERATING UNITS *(if any)*
MNB,DIR,NINDS (Drs. M. Hallett, J. Grafman, M. Dalakas); NB,DIR,NINDS (Dr. J. Alger); CNB,DIR,NINDS (Dr. J. Higgins); LCE,NHLBI (P. Jezzard); Univ. of Maryland, Medical School (Dr. H. Levin)

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Analytical Biometrics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.7	PROFESSIONAL: 1.8	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project undertakes the development and application of statistical methodology to neuroimaging. In particular, while brain imaging is a fundamental tool in neuroscience, the statistical treatment of the quantification of such images has lagged behind imaging technology. Numerous statistical problems are just beginning to be addressed in the analysis of neuroimages. These include: design of experiments to limit the search volume in image analysis by either *a priori* knowledge or a previous scan; the analysis of voxel (volume element) subtraction of images to investigate brain volumes of change in positron emission tomography (PET) or magnetic resonance imaging (MRI) scans of the same individuals under different tasks or drugs; multiple comparison issues to exploit the spatial correlation and to control the experiment-wise Type 1 error of any inference concerning a brain volume of apparent activity (where there may be 16,000 voxels per slice); techniques to analyze time-course data that is fundamental in functional MRI experiments; and the planning of experiments to ensure adequate power. Further, the resolution of these problems is all the more crucial as the imaging technology continues to improve dramatically. Research has been conducted concerning receiver operating characteristic (ROC) methodology that has direct application to the evaluation of different imaging modalities. Papers have been submitted, are in review or were published in FY94 on the following topics: computational and statistical tools for paired digital analysis, variability and covariability in magnetic resonance functional neuroimaging, and induced ischemia in the motor areas of the brains of normal volunteers as assessed by PET scans (MNB); new computationally-intensive methodologies to compare ROC plots that are useful in evaluation of imaging modalities, an investigation of quantitative MRI and clinical staging parameters for autosomal dominant cerebral ataxias (CNB); a functional MRI study on cortical activation during mental calculation (MNB); statistical methodology for analysis of functional MRI data; a statistical analysis based on estimation of the intrinsic temporal and spatial autocorrelations in the spectral domain for functional MR images in a study to assess the changes in the motor areas of the brains of normal volunteers that are activated by a finger movement task versus by its ideation only (MNB); and the variability of metabolites among normal volunteers using MR spectroscopy (NB).

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 NS 02810-05 BFSB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Coordinating Center for Collaborative Clinical Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jonas H. Ellenberg, Ph.D.	Acting Chief, Collaborative Studies Section	BFSB, DIR
Others:	Dallas W. Anderson, Ph.D.	Mathematical Statistician	BFSB, DIR
	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR
	Jack Panossian	Programmer	BFSB, DIR

COOPERATING UNITS *(if any)*
J. William Langston, M.D. and Caroline Tanner, M.D., Neurologists, California Parkinson's Foundation; Mario Melcon, M.D., Neurologist, Regional Hospital, Junin, Argentina

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Collaborative Studies Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.10	PROFESSIONAL: 0.10	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[x] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project encompasses all statistical coordinating center responsibilities for collaborative clinical studies undertaken by this Section and the Office of the Chief. A major initiative involves the study of the etiology of Parkinson's disease (PD) using the twin pair registry of the National Academy of Sciences/National Research Council. The prevalent cases of PD in the more than 6,000 twin pairs in which both members are alive, will be identified. This observational study will establish: environmental, medical and family histories of both affected and unaffected members of the twin pairs; DNA banking; and measurement of progression of disease over time. This project will investigate genetic and environmental contributions and their interactions to the etiology of PD. A Cooperative Agreement has been funded for the clinical aspects of this study. BFSB is acting as the statistical coordinating center. The study is ongoing.

A second collaborative project involves a two-phase prevalence survey of major neurologic disorders in Junin, a city of about 70,000 inhabitants, in the Province of Buenos Aires, Argentina. This household survey, funded by the Fundacion para la Investigacion en Neuroepidemiologia , is one of the largest of its kind in Latin America. On the basis of a two-stage systematic sample of households, 17,049 persons from 5,648 households were screened in phase 1, to identify those persons who possibly had a disorder of interest. In phase 2, the 817 persons screened as positive were evaluated (usually by means of a clinical examination) by neurologists using established diagnostic criteria. BFSB has collaborated on the design and data collection for this study, and is now collaborating on the data analysis and preparation of reports.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02717-09 CNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Catecholamine Metabolism in Health and Disease

P.I.:	Graeme Eisenhofer, Ph.D.	Visiting Associate	CNB, NINDS
Others:	Douglas Hooper, B.S.	Chemist	CNB, NINDS
	David Goldstein, M.D., Ph.D.	Chief, Clinical Neuroscience Section	CNB, NINDS

COOPERATING UNITS *(if any)*
Murray Esler, M.D., Gavin Lambert, B.S., Melbourne, Australia; Peter Friberg, M.D., Bengt Rundquist, M.D., Goteborg, Sweden; Jacques Lenders, M.D., The Netherlands

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Clinical Neurochemistry Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of this project are to quantify the pathways of catecholamine metabolism in experimental animals and humans and establish the involvement of disturbances in these pathways in certain disease processes. Tissue, plasma or urine samples are obtained before and during pharmacological or physiological manipulations and analyzed for concentrations of endogenous and exogenous radiolabelled catecholamines and their metabolites.

Findings in experimental animals are consistently demonstrating that neuronal pathways predominate over extraneuronal pathways for inactivation of the norepinephrine released by sympathetic nerves. A similar trend is emerging in ongoing studies in humans and swine where it is also possible to examine how the regional disposition of catecholamines is integrated among different organs and tissues. Of particular relevance the liver removes much of the norepinephrine, dopamine, and metabolites that are formed and released within the gastrointestinal tract, thereby hiding the true extent of the production of these catecholamines in mesenteric organs. The possibility that the much larger amounts of dopamine than norepinephrine metabolites produced in the lungs and gastrointestinal tract might reflect the existence of a third previously unknown non-noradrenergic catecholamine system is being actively explored. Findings in experimental animals that show how inhibition of the two A and B forms of monoamine oxidase cause different increases in O-methylated metabolites and decreases in the deaminated metabolites are being extended to studies of the involvement of these metabolizing enzymes in various neurological and behavioral disorders.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02839-04CNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Catecholaminergic Systems in Health, Stress, and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: David S. Goldstein, MD, PhD, Chief, CNS, CNB, NINDS

Others: A. Breier, ETB, DIR, NIMH; R.O. Cannon, III, CB, DIR, NHLBI; A. Golczynska, Visiting Associate, CNB, DIR, NINDS; G. Eisenhofer, Visiting Associate, CNB, NINDS; P. W. Gold, CNB, DIR, NIMH; C.Holmes, CMT, CNB, DIR, NINDS; D. Hooper, Chemist, CNB, DIR, NINDS; S G Kaler, Sr. Staff Fellow, HGB, DIR, NICHD, NIH; H. R. Keiser, HE, DIR, NHLBI; I. J. Kopin, Chief, CNB, DIR, NINDS; A. Quyyumi, CB, DIR, NIHLBI; J Stuhlmuller, Sr. Staff Fellow, CB, DIR, NHLBI; T. Yamamoto, Guest Worker, CNB, DIR, NINDS; P. Chang, Univ. Hosp., Leiden, Netherlands; J.P.M. Finberg, Technion, Haifa, Israel; P. Friberg, Sahlgrenska Hosp., Goteborg, Sweden; E. Grossman, Chaim Sheba Med. Ctr. Israel

COOPERATING UNITS *(if any)*
R. Kvetnansky, Slovak Acad. Sci., Slovakia; J.W.M. Lenders, Univ. Hosp. Nijmegen, Netherlands; K. Szemeredi, Egis Pharm., Hungary, J. Vernikos, NASA, Washington D.C., E. Wolfovitz, CNB, DIR NINDS

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Clinical Neurochemistry Section

INSTITUTE AND LOCATION
NINDS, NIH Bethesda, MD 20892

TOTAL STAFF YEARS: 3.7	PROFESSIONAL: 2.6	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our Section develops and applies methods for assessing the function of central and peripheral catecholaminergic systems and the coordination of these systems with other homeostatic systems in health, stress, and disease. Findings this year include: (1) positron emission tomographic (PET) scanning after systemic administration of 6-[^{18}F]fluorodopamine ([^{18}F]-6F-DA) provided a noninvasive, *in vivo* means to examine cardiac sympathetic innervation and function in humans, with acceptable absorbed radiation doses.(2) Clinical microneurographic and neurochemical methods were used to demonstrate glucocorticoid-induced sympathoinhibition in humans, to describe a distinctive neurochemical pattern in Menkes disease, to localize the site of sympathetic neuroeffector dysfunction in multiple system atrophy, to determine whether patients with Fabry's disease have sympathetic neuropathy, to test the "epinephrine hypothesis" of sympathetic neurotransmission, to document clozapine-induced noradrenergic stimulation, and to test for the presence of functional ß-adrenoceptors and angiotensin II receptors on sympathetic terminals in the human forearm. (3) Assays for plasma levels of O-methylated metabolites of catecholamines were developed and validated, and measurements of plasma levels of metanephrines were found to provide superior neurochemical means to diagnose pheochromocytoma, a catecholamine-secreting tumor. (4) Comprehensive measurements of concentrations of norepinephrine and dopamine and their metabolites in swine and sheep provided evidence for substantial production and metabolism of dopamine in porcine mesenteric organs and for non-neuronal catecholamine biosynthesis in ovine lungs. The renal DOPA-DA system is another "atypical" catecholaminergic system, and the possibility of abnormal function of this system in salt-sensitive hypertension is being explored, as well as treatment with L-DOPA as a renal dopaminergic prodrug in congestive heart failure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02870-03 CNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Amines: Regulation and Function With and Without Various Stressors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I. J. Kopin, M.D.	Chief, CNB, Director, DIR	CNB, NINDS
Others :	S. Al-Damluji, M.D.	Visiting Scientist	CNB, NINDS
	K. Pacek, M.D.	Visiting Fellow	CNB, NINDS
	Gal Yadid, Ph.D.	Visiting Fellow	CNB, NINDS
	J. Harvey-White, B.S.	Technician	CNB, NINDS
	D. Goldstein, M.D., Ph.D.	Medical Officer	CNB, NINDS
	R. Kvetnansky, M.D.	Visiting Scientist	CNB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Aminergic Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.9	4.0	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of this project are to determine the roles of catecholamines in brain functions that regulate motor activity, neuroendocrine secretion, and autonomic function in health, in disease states and in mediating responses to drugs in animals and in humans. *In vivo* microdialysis is used to monitor levels of monoamines and their metabolites in extracellular fluid in specific brain regions and has been combined with a parallel infusion cannula to introduce drugs or isotopically labeled compounds. Brain lesions are made surgically or by use of toxins specific to particular cell types. HPLC and liquid scintillation spectrophotometry are used in assays of materials in the microdialysates. Some studies is designed to examine the effects of a variety of stressors on brain amine release and simultaneously on levels of hormones in blood. In other studies and adrenal medullary tyrosine hydroxylase and the mRNA encoding this enzyme are assayed. In other studies, amine receptors and transporters have been examined in vitro using cells from different regions of brain and in cultured cell lines derived from the hypothalamus. Indirect evidence using blockade of serotonin (5-HT) reuptake or a neurotoxin (2,7-DHT) to destroy 5-HT neurons, suggests that 5-HT stimulates central neural DA release. Direct application of glycine also stimulates striatal DA release in conscious rats. Cerebrocortical tetrodotoxin-sensitive NE release is enhanced by chronic inhibition of monoamine oxidase A (MAO-A), but not type B. rats with unilateral striatonigral destruction with 6-OHDA, DA formation from locally perfused DOPA was less on the lesioned than on the intact side. Clorgyline (an inhibitor of MAO-A), augments DA production from DOPA both on the lesioned and the intact side, whereas deprenyl (an inhibitor of MAO-B) was without effect. Adult spontaneously hypertensive rats (SHRs) of the Okamoto strain had greater *in vivo* NE release and tyrosine hydroxylation in the posterolateral hypothalamus than normotensive rats of the same strain. Furthermore, juvenile SHRs had markedly greater responses of NE release and tyrosine hydroxylation in response to systemically administered yohimbine. The relationship of neuroendocrine responses to a variety of stressors (handling, immobilization, subcutaneous formalin injection, insulin, hemorrhage, or cold) evoke different patterns of responses. For example, insulin-induced hypoglycemia evoked marked, correlated increases in EPI and ACTH levels, whereas cold exposure increased plasma NE levels disproportionately compared with ACTH responses, and hypotensive hemorrhage increased ACTH levels disproportionately compared with catecholamine responses. Induction of rat adrenal tyrosine hydroxylase during immobilization stress was shown to be independent of pituitary hormones or innervation, suggesting a novel mechanism.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02910-01 CNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Distribution & Possible Function of Cannabinoid Receptors in the Brain & Periphery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	N.E. Buckley, Ph.D.	IRTA Fellow	CNB, NINDS
Others:	Eva Mezey, M.D., Ph. D.	Visiting Scientist	CNB, NINDS

COOPERATING UNITS *(If any)*
Andreas Zimmer, Ph.D., Visiting Associate, LCB, NIMH; Tom Bonner, Ph.D., Research Biologist, LCB, NIMH.

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Aminergic Mechanisms Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Marijuana has been used for many centuries as a psychoactive agent and also for its medicinal applications. For example, in humans, it has been used as an analgesic, anti-inflammatory, immunosuppressant and anticonvulsant. Binding studies with analogs of delta-9-tetrahydrocannabinol, the active component of marijuana (i.e., CP 55,940 etc.) have revealed that receptors for these ligands exist not only in the brain, but also in peripheral tissues (e.g., spleen, testis). To date, two cannabinoid receptors have been cloned, the central and peripheral cannabinoid receptor. We have used *in situ* hybridization to map the distribution of the peripheral cannabinoid receptor (CB2). This receptor was first discovered by Munro et in HL60 cells upon their differentiation into macrophages. Several *in situ* hybridization studies by different groups have suggested that mRNA for CB2 was present in the marginal zones of the spleen, but the cell types have not been identified. To further characterize the cell types in the spleen which may be responsible for binding cannabinoids, we have used specific immunocytochemistry in combination with in situ hybridization. We have also detected the mRNA for CB2 in testis, and are studying the hormonal regulation of mRNA expression in testosterone-treated young rats. In addition, we want to determine if there is a correlation between the mRNA level and a particular stage in spermatogenesis. Thus, we will synchronize spermatogenesis in rats using a vitamin A-deficient diet. It is known that vitamin A deprivation leads to the complete cessation of spermatogenesis in mammals, and that it is reversible upon supplementation with vitamin A. We have postulated that in the event that this receptor is missing, we could elucidate its function in a living organism. Thus, to study the significance of the presence of such a receptor, we will produce transgenic mice lacking the peripheral cannabinoid receptor. This gene will be targeted and mutated by homologous recombination. As a first step, we have successfully cloned the mouse CB2 receptor and characterized it by restriction endonuclease mapping. We have subcloned the fragments containing the coding region and those immediately flanking this region. We are presently mapping and sequencing these fragments to mutate the CB2 mouse gene in the plasmid construct and prepare it for homologous recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02883-02 CNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Distribution and Role of Neurotransmitter and Neuropeptide Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Eva Mezey, M.D., Ph. D.	Visiting Scientist	CNB, NINDS
Others:	Gabor Jakab, M.D.	Visiting Scientist	CNB, NINDS

COOPERATING UNITS *(if any)*
Drs. Susan Leeman & Harry Photoulakis, Boston University; Miklos Palkovits, M.D., Ph.D., Visiting Scientist, LCB, NIMH; Beth Hoffman, Ph.D., Senior Staff Fellow, LCB, NIMH.

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Aminergic Mechanisms Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.2	PROFESSIONAL: 3.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies on the neurotransmitter receptors in the gastrointestinal tract by using RNA probes to detect mRNA. We have determined that the H_2 receptor mRNA is present in immune cells of the lamina propria, and in the epithelium of the stomach and duodenum. We found that only about one-third of the parietal these cells bear the H2 receptor, while all mucus-containing cells in both the stomach and the duodenum synthesize the mRNA for the receptor. We have done similar double-labeling studies for the muscarinic, dopaminergic, and gastrin receptors, and are currently evaluating those results. We will soon have the result of our experiments to detect changes in the distribution of the above mRNAs in different stresses and in ulcerated stomachs. Among the gastrointestinal neuropeptides, we have performed several studies on CGRP, SP, and VIP.These peptides are present in both neuronal and cellular elements in the GI tract. CGRP-positive cells are often seen in close association with pepti-dergic peripheral sensory nerves, mainly in the duodenum. On the other hand, an intimate relationship between leukocytes and parietal cells was observed in the gastric glands suggesting their mediatory role in gastric acid secretion. Eosinophilic leukocytes have readily hybridized with two ^{35}S-labeled oligonucleotide probes with nonoverlapping sequences of rat CGRP gene. This phenomenon may reflect an interactive role of peptidergic afferent nerve fibers and immune cells in the protection of gastric mucosa against ulcers induced by chemical factors or stress.

We also begun to study the role of substance P (SP) in the pathogenesis of a toxin-mediated diarrhea and mucosal inflammation. Immune cells in the lamina propria contain SP and make its receptor. We have found a huge increase in SP receptor mRNA in both mucosal and lamina propria cells in the toxin-treated ileum compared to the control. At present, we are doing experiments to see how administration of an SP antagonist prior to toxin treatment might affect the invasion of inflammatory cells into the ileum. Using RNAse protection assay, we have demonstrated that there is a high amount of SP receptor mRNA in the small intestine.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02901-01 CNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of the Autosomal Dominant Cerebellar Ataxias

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joseph Higgins, M.D.	Chief, Clinical Neurogenetics Section	CNB, NINDS
Others:	Irwin J. Kopin, M.D.	Director, DIR	CNB, NINDS

COOPERATING UNITS *(if any)*

E, J Fitzgibbon, M.D., N. Patronas, M.D , CC; B. White, M.D., NIDDK; A. Pikus, NIDCD; M Polymero-poulos, M.D., NICHGR; L. Goldfarb, Ph.D., NINDS; N. Barton, M.D., Ph.D., NINDS; G. Campbell, Ph.D. , J. Grafman, Ph.D. , L.E. Nee, NINDS.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Clinical Neurogenetics Unit, Clinical Neuropharmacology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither

[x] (a1) Minors

[x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the genetic causes of the autosomal dominant cerebellar ataxias (ADCA) by linkage analysis and to define intra- and interfamilial phenotypic variation and disease progression by serial measurements of biochemical, electrophysiologic, clinical and radiographic parameters. The following issues will be addressed in this study: (1) Is there true genetic heterogeneity or are there other influences that cause the clinical variation in ADCA? (2) Since monoaminergic metabolism is altered in ADCA, how do these abnormalities correlate with disease severity? (3) Can quantitative MRI abnormalities be correlated with the ACRS? (4) Can the addition of psychometric and neurophysiologic test results with ACRS scores and functional stages yield a comprehensive and clinically useful genetically-based staging classification? (5) Can a longitudinal prospective study monitoring these parameters yield a staging classification with enough statistical power to detect changes in the natural progression of the disease during therapeutic drug trials?

Results of studies demonstrate a relationship between subscores on the Ataxia Clinical Rating Scale (ACRS) and the midsagittal areas of the cerebellum, pons and cervical spinal cord on quantitative MRI. Low levels of CSF HVA, a dopamine metabolite, are found in ADCA patients and is related to the degree of pontine atrophy on MRI. In contrast to other studies, 5-HIAA levels were found to be normal when compared to age-matched controls. Although the low levels of CSF HVA probably reflect neuronal loss, the possibility of a metabolic block in dopamine metabolism is being investigated.

Genetic screening of 77 patients from 24 families in the Middle Atlantic States Region with ADCA for a trinucleotide repeat expansion on chromosome 6p identified 3 individuals from 2 families with this abnormality. A locus for ADCA was mapped to chromosome 14q24.3-qter in one family and other large kindreds are being tested for candidate loci.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02902-01 CNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Biotin-Responsiveness in Familial Pyruvate Carboxylase Deficiency

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joseph Higgins, M.D.	Chief, Clinical Neurogenetics Section	CNB, NINDS

COOPERATING UNITS *(if any)*
Anita Pikus, M.A., NOB, NIDCD; Michalis Polymeropoulos, M.D., NICHGR; Lev Goldfarb, Ph.D., NINDS; Jordan Grafman, Ph.D., NINDS; Linda E. Nee, M.S.W., NINDS; Susumu Sato, M.D., NINDS

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Clinical Neurogenetics Unit, Clinical Neuropharmacology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[x] (a1) Minors
[x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to document genotypic differences and biotin-responsiveness in family pedigrees with pyruvate carboxylase deficiency. Brainstem and diffuse subcortical white matter changes are found on MRI in several members of this kindred. Improvement in myelination is found on serial MRI after one child received biotin therapy. The pathophysiologic basis underlying these myelin changes may involve PC's role in providing citrate and cytosolic acetyl Co-A as sources for fatty acid synthesis. It is not clear whether the improved myelination in this patient is a consequence of early biotin treatment or simply reflects the natural course of this disease. His parents' fibroblast PC enzyme activity (55-65% of the mean of controls) was consistent with that predicted for obligate heterozygotes. There was a small increase in PC activity in the parent's lymphocyte PC activity. The genetic sequence of the biotin binding domains in all family members appear normal. Studies are in progress to define the mutations in the PC gene responsible for this disorder.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02903-01 CNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Use of Attenuated Hepatitis A as a Vector for Neurogene Transfer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joseph Higgins, M.D.	Chief, Clinical Neurogenetics Section	CNB, NINDS
Others:	Susan A. Zullo, Ph.D.	Pre-IRTA	CNB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Clinical Neurogenetics Unit, Clinical Neuropharmacology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this research project is to design and test the safety and efficacy of the attenuated hepatitis A strain HM175 (HAV) as a vector for gene transfer into cultured human fetal brain tissue and into the CNS of nonhuman primates. This project involves cloning expression vectors for neurogenes by recombinant techniques and testing their infection efficiency as well as their transcription and translation ability. Studies comparing the tropism of HAV for neuronal and glial elements will be carried out in cultured human fetal spinal cord and brain.

Studies have indicated that HAV can efficiently infect cultured human fetal brain tissue without noticeable cytopathic effects. Direct inoculation of HAV into the spinal cord and thalamus of *Macaca mulatta* did not cause any significant clinical or histologic effects. The HAV negative strand replicative intermediate was detectable by RT-PCR in the brain up to four weeks after direct inoculation. RT-PCR *in situ* is being developed to determine the cellular location of the HAV negative strand replicative intermediate. Plasmid vectors containing the full length cDNA of HAV are grown in *E. coli* competent cells (strain DH5α) and picomaviral genetic elements, reporter genes and human cDNAs are being inserted into known loci within the HAV genome that do not effect the virus' viability.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 NS 02630-11 CNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical, Genetic and Biochemical Studies of Familial Alzheimer's Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Interim P.I.:	L.E. Nee, M.S.W.	Social Science Analyst	OCD, CNP, NINDS

COOPERATING UNITS *(if any)*
Lev Goldfarb, M.D., NINDS; Jordon Grafman, Ph.D., NINDS; Jay Robbins, M.D., NCI

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Clinical Neuropharmacology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects   [X] (b) Human tissues   [ ] (c) Neither
[X] (a1) Minors
[X] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

With the departure of the previous PI, this project has been maintained mainly as a resource for future research to be developed in relation to a new Unit on Clinical Neurogenetics which has been established in the CNB. Inherited Alzheimer disease - longitudinal data collection, expansion of pedigree information and recruitment of additional family members, as well as counseling of numerous families, some followed since 1977, has continued. Collaborations have also continued although no patient was admitted during the past year. In September 1994, we hope to admit families with the continued collaboration of Trey Sunderland, M.D., NIMH. Longitudinal study involves LP's, PET, DNA, psychological testing, and psychological and genetic counseling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02752-07 CNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Synthesis and Expression of Neurotrophic Agents and Neuropeptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joan Schwartz, Ph.D.	Chief, Molecular Genetics Section	CNB, NINDS
Others:	Emil Viskupic, Ph.D.	Visiting Associate	CNB, NINDS
	Dahlia Minc-Golomb, Ph.D.	Visiting Fellow	CNB, NINDS
	Takayuki Taniwaki, M.D.	Visiting Fellow	CNB, NINDS
	Yukihiro Sugita, M.D., Ph.D.	Visiting Associate	CNB, NINDS
	Vivian Wu, Ph.D.	Visiting Fellow	CNB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 5.8	PROFESSIONAL: 5.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evidence suggests that parallel biochemical and regulatory processes occur during normal development and following various forms of central nervous system (CNS) injury. Among these areas of particular interest are: (1) identification of CNS neurotrophic factors; and (2) the analysis of the regulation of neurotrophic factor and neuropeptide gene expression during development and in response to injury. Studies are underway to identify trophic factors produced in specific model systems, since recent evidence suggests that a family of nerve growth factors (NGF) exists, each specific for certain populations of neurons. 6-OHDA lesioned rats represent a Parkinsonian-like model in which changes in NGF and the related neurotrophic factors BDNF (brain-derived neurotrophic factor) and NT-3 are being examined at the level of mRNA, protein, and biologic activity. Since astrocytes can synthesis a number of neurotrophic factors, primary cultures of astrocytes are used to determine factors which regulate production of these potential trophic factors. Reactive astrocytes are prepared from regions affected by the various injuries and their production of trophic factors compared to that of control astrocytes. Potential neurotrophic functions for the neuropeptides, enkephalin and somatostatin, in early CNS development have been demonstrated in culture and in transgenic mice: enkephalin acts as a negative modulator of CNS development while somatostatin is a positive trophic factor. Cytokines can induce expression of trophic factors as well as of nitric oxide synthase: since microglia activated by brain injury are being examined. Analysis of a neurologic syndrome found in 60% of transgenic mice expressing an antisense interleukin-3 construct has demonstrated a cellular lesion in the cerebellar peduncle. A retinal pigment epithelium-derived factor (PEDF) functions as a survival factor for cerebellar granule cells.

PHS 6040 (Rev 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02218-19 CNB

PERIOD COVERED

October 1,1993 to September 30,1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Sources and Effects of Reactive Oxygen Intermediates in the Brain

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Gilbert, Ph.D.	Research Physiologist	BS, NINDS
Others:	J. Snell	Biologist	BS, NINDS

COOPERATING UNITS (*if any*)

Georgetown University, Washington, D.C. (C. A. Colton, O. Chernyshev and F. Pagan)

LAB/BRANCH

Clinical Neuroscience Branch, DIR

SECTION

Biophysics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Experiments have been performed on <u>microglia</u> and <u>astrocytes</u> in the <u>central nervous system</u> cultured from cerebral cortices of rat and hamsters. We have previously shown that activated microglial rat cells produce the <u>superoxide radical anion,</u> a <u>reactive oxygen species (ROS)</u>. ROS include also the hydroxyl radical, hydrogen peroxide, and nitric oxide. Activated rat microglia also produce nitric oxide. However, the release of nitric oxide does not significantly increase until after about 10 hours of stimulation, whereas the release of the superoxide radical anion is completed within 2 to 3 hours of stimulation. Also, hamster microglia produce more than ten times the amount of the superoxide radical anion produced by rat microglia. In all the conditions we have tested, there is no evidence that the hamster microglia releases the nitric oxide. In another set of experiments, we have shown that N-acetyl-cysteine increases the survival of tyrosine hydroxylase-positive rat mesencephalic neurons grown in tissue culture. In addition, there are more surviving rat mesencephalic neurons at an oxygen pressure of 38 torr than at the commonly used oxygen pressure of 150 torr.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02709-09 CNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Secretion of Neurotransmitters and Hormones

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	G. Ehrenstein, Ph.D.	Chief, Biophysics Section	CNB, NINDS
Others:	M. Jia, M.D.	Visiting Associate	CNB, NINDS
	A. Mbuyi-Kalala, D.Sc.	Visiting Associate	CNB, NINDS

COOPERATING UNITS (*if any*)

LAB/BRANCH

Clinical Neuroscience Branch, DIR

SECTION

Biophysics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.8	PROFESSIONAL: 3.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We have found calcium channels in parathyroid cells that have a single-channel conductance of about 0.6 pS under normal physiological conditions. Independent of membrane potential or calcium concentration, the channels are open about 8% of the time. These channels insure a strong correlation between intracellular and extracellular calcium concentrations. Such a correlation is important to insure proper functioning of parathyroid cells, since the intracellular calcium concentration controls secretion of parathyroid hormone (PTH), and secretion of PTH, in turn, affects the extracellular calcium concentration. We have determined the effect of fragments of PTH on measurements of PTH secretion. We measured the amount of intact PTH in solution by radioimmunoassay (RIA). We then added known quantities of PTH fragments, and used RIA to remeasure the amount of intact PTH. The presence of the fragments had a significant impact on the apparent quantity of intact PTH measured by RIA. Depending on the specific concentrations of the several molecules involved, the apparent quantity of intact PTH sometimes appeared to increase and sometimes appeared to decrease. The experimental results could be simulated by a model wherein fragments can not only bind to antibody, but can also bind to intact PTH, resulting in a reduced affinity for antibody.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS 02905-01 CNB

PERIOD COVERED
October 1,1993 through September 30,1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of β-Amyloid on Ion Transport Across Cell Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Ehrenstein, Ph.D. Chief, Biophysics Section CNB, NINDS

COOPERATING UNITS *(if any)*
Laboratory of Neurosciences, NIA, NIH (Z. Galdzicki, R. Fukuyama, N.C. Wadhwani, S.I. Rapoport)

LAB/BRANCH
Clinical Neuroscience Branch, DIR

SECTION
Biophysics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda,, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since β-amyloid is a putative causal agent for Alzheimer's disease (AD), we have examined some of its effects on ion transport across cell membranes. Incubation of PC-12 cells with β-amyloid for 24 hours caused a significant increase in the choline conductance of the cells. If a similar effect occurs in cholinergic neurons of AD patients, the leakage of choline out of the neurons could reduce the intracellular concentration of choline and result in decreased synthesis and secretion of acetylcholine. This could explain the reduced concentration of acetylcholine in the brains of AD patients.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 00815-34 DMNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of Complex Lipids of Nervous Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	P.G. Pentchev, Ph.D.	Section Chief	DMNB, NINDS
Others:	R.O. Brady, M.D.	Chief	DMNB, NINDS
	J.M. Quirk, M.S.	Biochemist	DMNB, NINDS
	C. Roff, Ph.D.	Spec. Expert	DMNB, NINDS
	E. Goldin, Ph.D.	Visiting Fellow	DMNB, NINDS
	M. Comly, B.S.; A. Cooney	Biologists	DMNB, NINDS
	E.D. Carstea, Ph.D.	Senior Staff	DMNB, NINDS

COOPERATING UNITS *(if any)*
LCDB, NIDDK; Lab. Biochem., Fac. Med., Lyon-Sud, France

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics, Molecular and Cellular Pathophysiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.32	2.18	5.14

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Types C and D Niemann-Pick disease are characterized by abnormal intracellular cholesterol homeostasis. The molecular lesion in these disorders causes: (1) failure to down-regulate LDL receptors on cell membranes; (2) lack of down-regulation of HMGCoA reductase, a key enzyme in cholesterol biosynthesis; and (3) inability to up-regulate acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the esterification of intracellular cholesterol. Tests have been developed and are now widely used in medical practice for the diagnosis of Types C and D Niemann-Pick disease, identification of heterozygotes, and the prenatal diagnosis of these conditions.

We have linked the NP-C mutation to chromosome 18 by positional cloning. We have obtained partial correction of the defective cholesterol metabolism with YACs within the NP-C defined internal on chromosome 18 q11. Identification of the gene will enable us to assess direct DNA diagnosis and the initial protein and gene replacement studies. The Golgi apparatus has been shown to regulate lysosomal cholesterol transport. Characterization of the cholesterol transporter as identified by the NP-C mutation will provide the tools to begin to delineate the molecular mechanisms as well as cellular pathways of intracellular cholesterol transport. Armed with such information, we will study cholesterol processing in normal cells and in pathogenic conditions represented, not only by the NP-C cell, but also by other cholesterol lipidodic states such as the atherogenic foam cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02162-20 DMNB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis of Compounds Analogous to Sphingolipids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S.P. Miller, Ph.D.	Spec. Expert	DMNB, NINDS
Others:	A. Boumendjel, Ph.D.	Visiting Fellow	DMNB, NINDS

COOPERATING UNITS *(if any)*
Biochemistry and Molecular Biology Department, Georgetown University Medical Center, Washington, D.C.

LAB/BRANCH
Developmental and Metabolic Neurology

SECTION
Neurochemical Methology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.02	1.02	0

CHECK APPROPRIATE BOX(ES)
- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Project terminated - PI has left NIH.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02163-20 DMNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Development of Analytical Methods for Use in Research on Sphingolipidoses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)
PI: S. P.Miller, Ph.D. Special Expert DMN NINDS

COOPERATING UNITS (*if any*)

LAB/BRANCH
Developmental and Metabolic Neurology

SECTION
Neurochemical Methodology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Project Terminated. PI left NIH.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02453-14
DMNB

PERIOD COVERED
October 1,1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Gaucher's Disease: Biochemical and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	N. Barton, M.D., Ph.D.	Section Chief	DMNB, NINDS
Others:	R. O. Brady, M.D.	Chief	DMNB, NINDS
	G. Murray, Ph.D.	Special Volunteer	DMNB, NINDS
	G. Zirzow, B.S.	Biologist	DMNB, NINDS
	K. Oliver, M.S.	Biologist	DMNB, NINDS
	R. Schiffmann,M.D.	Visiting Associate	DMNB, NINDS
	C. Parker, M.D.	Clinical Associate	DMNB, NINDS
	T. Banerjee, M. D.	Visiting Associate	DMNB, NINDS

COOPERATING UNITS (if any)
Massachusetts Gen. Hospital, Dept. of Orthopedic Surgery, Boston, MA: (H. Mankin, D. Rosenthal, S. Doppelt); Children's Hospital, Washington, D. C. (P. Guzetta)

LAB/BRANCH
Developmental and Metabolic Neurology

SECTION
Clinical Investigations & Therapeutics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.29	3.19	4.10

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
Extraordinarily gratifying success has been obtained with enzyme replacement therapy in patients with Gaucher's disease. All patients who received macrophage-targeted human placental glucocerebrosidase had significant clinical benefit. The hemoglobin level rose in all patients, and within six months after initiation of therapy, the size of the spleen had decreased in all recipients. Long-term treatment was required to produce reversal of skeleton pathology. Patients who received the enzyme were able to resume activities such as work or school that they had been unable to carry out before enzyme replacement. The U.S. Food and Drug Administration has approved the use of macrophage-targeted glucocerebrosidase as specific therapy for patients with Type 1 Gaucher's disease. The beneficial effect of enzyme replacement in patients with Gaucher's disease has been repeatedly confirmed by many independent investigators. The quantity of enzyme that patients require to be maintained in good health is far less than that which is initially necessary to reverse the clinical and pathological manifestations of the disorder. Patients with milder clinical signs of the disorder improve with smaller amounts of enzyme than that required by more severely affected individuals. Recombinantly produced macrophages targeted glucocerebrosidase has been found to be as effective as the placental enzyme used in the original clinical efficacy trial.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02664-10 DMNB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Studies of Neurogenetic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Barton, M.D., Ph.D.	Section Chief	DMNB	NINDS
Others:	R. Brady, M.D.	Chief	DMNB	NINDS
	C. Parker, M.D.	Clinical Associate	DMNB	NINDS
	R. Schiffmann, M.D.	Visting Associate	DMNB	NINDS
	M.A. McKee, M.D.	Clinical Associate	DMNB	NINDS
	T. Banerjee, M.D.	Visiting Associate	DMNB	NINDS
	P. Pentchev, Ph.D.	Section Chief	DMNB	NINDS

COOPERATING UNITS *(if any)*
Neuroimaging Branch, NINDS, and Laboratory of Molecular and Cellular Neurobiology, NINDS

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.69	PROFESSIONAL: 2.29	OTHER: 0.40

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have identified a novel hereditary demyelinating disorder and documented highly specific magnetic resonance spectroscopic aberrations in these patients.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02731-08
DMNB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Gene Therapy of Inherited Enzyme Deficiencies

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Karlsson, M.D., Ph.D.	Acting Chief, M&MG	DMNB	NINDS
Others:	M. Makoto, M.D.,Ph.D	Special Volunteer	DMNB	NINDS
	S. Klupfel-Stahl	Special Volunteer	DMNB	NINDS
	M. Blanco,B.S.	Special Volunteer	DMNB	NINDS
	R. Brady, M.D.	Chief	DMNB	NINDS
	R. Schiffmann, M.D.	Visiting Associate	DMNB	NINDS
	M. Amiri,MS	Special Volunteer	DMNB	NINDS
	L. Xu, M.D., Ph.D.	Visiting Associate	DMNB	NINDS

COOPERATING UNITS (*if any*)
Clinical Hematology Branch, NHLBI (Drs. R. Donahue and C. Dunbar)

LAB/BRANCH
Developmental and Metabolic Neurology

SECTION
Molecular and Medical Genetics

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.12	4.82	3.30

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)
Gaucher's disease is an inherited disorder caused by a mutation of the gene for the enzyme glucocerebrosidase. The normal gene for this enzyme has been cloned by several laboratories. We have constructed high-titer, helper-free recombinant retroviruses containing this gene. We have shown that infection of cell lines from normal individuals and patients with Gaucher's disease with this retroviral vector results in increased glucocerebrosidase activity. The glucocerebrosidase gene has been transferred efficiently into progenitor cells and repopulating stem cells of mouse bone marrow, and is expressed at the RNA and protein level in the progeny of CFU-S multipotential progenitor cells following gene transfer. The gene has also been transferred efficiently into murine hematopoietic stem cells that can be used to repopulate secondary transplant recipients. The vector genome can be detected in all hematopoietic lineages and produces human glucocerebrosidase RNA in all hematopoietic tissues tested.High levels of human glucocerebrosidase are generated in hematopoietic tissues. The macrophages of these long-term reconstituted mice produce human glucocerebrosidase levels that are equivalent to the endogenous mouse enzyme levels. Hematopoietic stem cells from rhesus monkeys have been transduced by glucocerebrosidase vector supernatants with a marking efficiency of approximately 1%. The human glucocerebrosidase gene has been introduced into human hematopoietic progenitor cells with a high degree of efficiency. Vector-transduced hematopoietic progenitors from Gaucher's patients produce progeny cells with glucocerebrosidase enzyme values similar to those of normal individuals. A clinically acceptable supernatant infection protocol has recently been developed which can be used to correct the enzyme deficiency in hematopoietic cells from Gaucher patients following gene transfer into primitive hematopoietic cells. The recombinant DNA Advisory Committee has approved the first clinical protocol using these vectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02771-06 DMNB

PERIOD COVERED
October 1,1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Modification of Growth Factor Genes by Gene Targeting

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Kulkarni, Ph.D.	Senior Staff Fellow	DMN	NINDS
Others:	S. Karlsson, M.D., Ph.D.	Acting Section Chief	DMN	NINDS
	L. Yaswen, Ph.D.	IRTA	DMN	NINDS
	J. Hallenbeck, M.D.	Chief	SB	NINDS
	C.-G. Huh,Ph.D.	IRTA	DMN	NINDS
	S. Yuspa, M.D.	Chief	LCCTP	NCI
	A. Roberts, Ph.D.	Deputy Chief	LC	NCI
	J.. Keller, Ph.D.	Senior Staff Fellow	BRMP	NCI

COOPERATING UNITS *(if any)*
Stroke Branch, NINDS; LCCTP, NCI, Frederick Cancer Research & Development Center, NCI

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Molecular and Medical Genetics

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0.85	0.85	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gene targeting by homologous recombination can be used to activate or inactivate cellular genes in eukaryotic cells. The objective of this project is to alter the functional status of genes that control growth and maturation of specific tissues and study the biological consequences of these molecularly defined alterations to delineate specific roles of transforming growth factor beta-1 (TGF-beta 1). We have generated TGF-beta 1 knockout mice using gene targeting technique. These mice are deficient in this growth factor. After normal growth for the first two weeks, they develop a rapid wasting syndrome and die as early as three weeks of age. Histopathological examination revealed multifocal inflammatory response with massive infiltration of lymphocytes and macrophages in many organs, but primarily in heart and lungs. Further examination indicated that onset of multifocal inflammation follows elevated expression of major histocompatibility class I and II genes and increased adhesion of leukocytes to endothelium of blood vessels in the affected tissues. Increased adhesion of leukocytes in these animals was associated with higher expression of VLA4 cell surface markers in the leukocytes. Fibronectin peptide treatment of the knock-out mice blocked inflammation, and moderated the body weight and restored homeostatic regulation of immune cell proliferation and inflammation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02816-05 DMNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis of Inhibitors of N-Myristoyltransferase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S.P. Miller, Ph.D	Special Expert	DMN	NINDS
Others:	K.M.Neder, Ph.D	IRTA Fellow	DMN	NINDS
	S. A. French, B.S.	Chemist	DMN	NINDS

COOPERATING UNITS *(if any)*
LMB, DTTD, FDA; Cell Signaling and Oncogenesis Group, CPB, NCI

LAB/BRANCH
Developmental and Metabolic Neurology

SECTION
Neurochemical Methodology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.28	0.47

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated. P.I left NIH.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02843-03 DMNB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigation of the Etiology of Mucolipidosis IV

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.O. Brady, M.D.	Chief	DMNB NINDS
Others:	E.Goldin, Ph.D.	Visiting Fellow	DMNB NINDS
	P.G. Pentchev, Ph.D.	Section Chief	DMNB NINDS
	N.W. Barton, M.D., Ph.D.	Section Chief	DMNB NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Developmental and Metabolic Neurology

SECTION
Cellular and Molecular Pathophysiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.20	1.05	0.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
We have developed and validated a novel test for the prenatal diagnosis of mucolipidosis IV. The phenotypic alteration that permitted the development of this test is being used as a marker for the functional cloning of the gene that is mutated in mucolipidosis IV.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02844-03
DMNB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigation of the Etiology of Batten's disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Calvin F. Roff, Ph.D.	Special Expert	DMNB NINDS
Others:	Peter G. Pentchev, Ph.D.	Section Chief	DMNB NINDS
	Roscoe O. Brady, M.D.	Branch Chief	DMNB NINDS

COOPERATING UNITS *(if any)*
Section on Receptor Biochemistry and Molecular Biology, NINDS

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Molecular and Cellular Pathophysiology

INSTITUTE AND LOCATION
NINDS,NIH, Bethesda, MD. 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0.73	0.73	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated. PI has left NIH.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02845-03 DMNB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigation of Enzyme Replacement Therapy in an Analogue of Human GM_1 Gangliosidosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R. O. Brady, M.D.	Chief	DMNB NINDS
Others:	G.J. Murray, Ph.D.	Special Volunteer	DMNB NINDS
	J.M. Quirk, M.S.	Biochemist	DMNB NINDS

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, NINDS

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.10	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Enzyme replacement therapy has been shown to be extraordinarily effective for patients with Type 1 (non-neuronopathic) Gaucher's disease. We now need to develop procedures to deliver useful amounts of enzymes to the brain in patients with hereditary metabolic storage disorders. We are examiningthe effect of human placental beta-galactosidase on the amount of ganglioside GM1 in animal analogues of human generalized (GM1) gangliosidosis using a new intracerebral protein delivery system.We are also determining the distribution of glucocerebrosidase in the brain using convection-enhanced intracerebral injection of this enzyme.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02878-02 DMNB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Animal Models for Genetic Defects.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S. Karlsson, M.D.,Ph.D.	Acting Chief, M&MGS	DMNB	NINDS
Others :	C.-G. Huh, Ph.D.	IRTA Fellow	DMNB	NINDS
	J. Higgins, M.D.	Medical Staff Fellow	DMNB	NINDS
	A. Kulkarni, Ph.D.	Senior Staff Fellow	DMNB	NINDS

COOPERATING UNITS *(if any)*
P. Loh, NICHD, NIH

LAB/BRANCH
Developmental and Metabolic Neurology

SECTION
Molecular and Medical Genetics

INSTITUTE AND LOCATION
NINDS,NIH,Bethesda, MD. 20892

TOTAL STAFF YEARS: 1.34	PROFESSIONAL: 1.25	OTHER: 0.09

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gene targeting in embryonic stem (ES) cells is being used to inactivate (knock-out) genes and use the mutated ES cells to generate mice with a mutation at the targeted locus. We have targeted the pro-opioelanocortin (POMC) gene in embryonic stem cells in order to determine the influence of POMC during development and post-natally. The POMC-targeted ES cells are now being used to generate chimeric animals that may carry the gene defect in the germline. Similarly, the cystatin C gene has been cloned and gene targeting constructs have been made in order to inactivate the cystatin C gene in ES cells. Cystatin-C mice will be made and the muated human cystatin C gene from a patient with hereditary cerebral angiopathy will be introduced into one of the constructs in order to make a mouse model for hereditary stroke.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02893-01 DMNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Modification of Brain-Specific Chyclin-Dependent Kinase Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	A. Kulkarni, Ph.D.	Unit Chief	DMNB	NINDS
Others:	T. Oshima, M.D. Ph.D.	Visiting Fellow	DMNB	NINDS
	G. Longnecker, B.S.	Biologist	DMNB	NINDS
	H. Pant	Section Chief	LNC	NINDS

COOPERATING UNITS *(if any)*

LNC, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40	0.18	0.22

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Members of cdc2 family of protein kinases are known for their pivotal role in the regulation of the eukaryotic cell cycle. The potential roles of neuronal specific cdc2-like kinases in stabilizing neurofilament skeleton and in axonal morphogenesis through tau by phosphorylation are not well delineated. The main objectives of this project are: (1) to disrupt the genomic locus of neuronal cdc2-like kinase (cdk5) in embryonic stem cells, and then use the targeted cells to generate mouse models to study *in vivo* function of these kinases; and (2) to overexpress cdk5 in neuronal cell lines and *in vivo* in mice. Toward achieving these objectives, we have isolated 8 genomic clones of cdk5 from the 129/svj library. These isogeneic clones have been partially characterized to define intron/exon boundaries and exonic sequences. A gene-targeting construct has been engineered with a deletion of exons 3 through 5 and insertion of neomycin resistance gene at the site of deletion. At the 3' end of the construct, the herpes virus thymidine kinase gene is included for positive/negative selection using G418 and gancyclovir. We have also isolated a murine cDNA clone using the RT-PCR technique, and have obtained complete nucleic acid sequence data. Gene targeting experiments in ES are currently in progress to obtain targeted clones.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02894-01 DMNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Generation of Mouse Models of Neurological Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Kulkarni, Ph.D.	Unit Chief	DMN	NINDS
Others:	K. Yoshida, M.D., Ph.D.	Visiting Fellow	DMN	NINDS
	T. Oshima, MD, Ph.D.	Visiting Fellow	DMN	NINDS
	L. Yaswen, Ph.D.	IRTA Fellow	DMN	NINDS
	G. Longnecker, BS	Biologist	DMN	NINDS
	C. Murray, Ph.D.	Special Expert	DMN	NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS,NIH,Bethesda,MD 20892

TOTAL STAFF YEARS: 1.87	PROFESSIONAL: 1.65	OTHER: 0.22

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gene knock-out mouse models have become gold standards for delineating molecular and functional roles of specific genes. In an attempt to generate such models for neurological disorders, we have initiated studies to disrupt apolipoprotein D (ApoD) gene in mouse embryonic stem cells. We have used rat ApoD cDNA probe to screen the 129/svj mouse genomic library. Eight genomic clones of ApoD have been isolated and partially characterized. These isogeneic clones will be used to make targeting vectors for gene disruption.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02318-17 ERB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Pharmacology of Antiepileptic Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William H. Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	Barbara Stertz, RN	Nurse,CES	ERB	NINDS
	Beth Malow, M.D.	Clinical Associate (SF), CES	ERB	NINDS
	David Ko, M.D.	Visiting Associate (CA), CES	ERB	NINDS

COOPERATING UNITS *(if any)*

Office of the Clinical Director, NINDS

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.25	3.25	1.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[x] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We investigated the effects of rate of carbamazepine (CBZ) taper and CBZ level on seizure type and frequency in patients being withdrawn from antiepileptic drugs (AEDs). Rapid taper (4 days) led to significantly more generalized tonic-clonic seizures than slow taper (10 days). Neuropsychiatric disorders such as panic were increased during drug withdrawal.

We have conducted several double-blind placebo-controlled trials of felbamate (FBM), an experimental antiepileptic drug (AED), in patients with complex partial seizures and the Lennox-Gastaut syndrome, a severe childhood epileptic encephalopathy. In the most recent study, we evaluated the effect of FBM monotherapy on seizure rate in patients undergoing presurgical monitoring. All their AEDs were withdrawn, and they were drug-free for 5 ± 2.4 days before randomization to FBM or placebo. After a 4-day titration, seizures were counted for 14 days. Eight of 19 placebo patients randomized to placebo, compared to 13 of 21 on FBM, completed the 18-day study. Two FBM patients dropped out due to seizures, and 6 due to side effects, including anxiety, difficulty sleeping, abdominal discomfort, acute psychosis and orobuccal dyskinesia. Ten placebo patients met the criteria for premature termination due to seizures, and one had an episode of panic. Six of 11 placebo, and 4 of 8 FBM dropouts occurred during titration. Patients on FBM had significantly lower seizure rates, whether all randomized patients, patients who survived titration, or study completers were compared. Our trial design allowed us to collect information on both seizure reduction and drug toxicity more effectively than in conventional trials.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02772-07 ERB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Uncompetitive NMDA Antagonists as Anticonvulsants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Rogawski, M.D., Ph.D.	Chief, NES	ERB	NINDS
Others:	Shun-ichi Yamaguchi, Ph.D	Psychologist, NES	ERB	NINDS
	Tushar Kokate, Ph.D.	Visiting Fellow, NES	ERB	NINDS
	Swaminathan Subramaniam, M.D., Ph.D.	Visiting Associate, NES	ERB	NINDS
	Duan-chan Uyakul, Ph.D.	Lab. Analytical Chemistry		NIDDK
	Lewis K. Pannell, Ph.D.	Lab. Analytical Chemistry		NIDDK

COOPERATING UNITS *(if any)*
Neurogen Corporation, Branford, CT

LAB/BRANCH
Epilepsy Research Branch, CNP, DIR

SECTION
Neuronal Excitability Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 1.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

ADCI (5-aminocarbonyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10- imine), a low-affinity uncompetitive NMDA antagonist, is a broad-spectrum anticonvulsant with a favorable side effect profile. However, the drug's clinical utility will be dependent upon its ability to maintain efficacy when it is administered to patients on a chronic basis. Therefore, we sought to determine if tolerance develops to the anticonvulsant activity of ADCI using the maximal electroshock (MES) test to assess seizure protection. Mice were treated with three daily injections of a 2 x ED_{50} dose for MES protection (18 mg/kg, i.p.) or vehicle for 7 or 14 days. On the day after the chronic treatment protocol, all animals received a challenge dose of ADCI (18 mg/kg) and 15 min later were evaluated in the MES test. In control animals, 83-94% of animals were protected and the ADCI plasma levels immediately after the MES test were 5.5-9.7 µg/ml. In treated animals, 29% and 0% of animals were protected at 7 and 14 days, respectively, and the ADCI plasma levels were 77% and 52% of control. [^{3}H]Dizocilpine binding to brain NMDA receptors was unaltered by the chronic drug treatment. In subsequent experiments, we determined that 14-day chronically treated animals could be completely protected by increased doses of ADCI (ED_{50}, 28.9 mg/kg). In both naive and chronically treated animals receiving a challenge dose of ADCI, ADCI plasma levels declined in two phases, the first with time constant of ~55 min and the second with a much slower rate. The estimated plasma concentrations of ADCI reflecting threshold (3-5 µg/ml) and 50% protection (5-7.5 µg/mg) were similar in naive and chronic animals. We conclude that tolerance to ADCI is due to pharmacokinetic factors (enhanced first-pass metabolism) and does not result from a reduction in anticonvulsant efficacy.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02236-19 ERB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diagnostic and Therapeutic Reevaluation of Patients With Intractable Epilepsy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William H. Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	Susumu Sato, M.D.	Chief, EEG Lab	OCD	NINDS
	Charles DeCarli, M.D.	Clinical Associate (CO),CES	ERB	NINDS
	Teresa Blaxton, Ph.D.	Staff Fellow, CES	ERB	NINDS
	Laroy Penix, M.D.	Senior Staff Fellow, CES	ERB	NINDS

COOPERATING UNITS *(if any)*
EEG Laboratory, Office of The Clinical Director, NINDS

LAB/BRANCH
Epilepsy Research Branch, CNP, DIR

SECTION
Clinical Epilepsy Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.9	PROFESSIONAL: 2.1	OTHER: 1.8

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[x] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Clinical Epilepsy Section is using a multimodality approach, with an emphasis on neuroimaging techniques, including positron emission tomography (PET) and magnetic resonance imaging (MRI),to evaluate patients with severe epilepsy. PET uses radiolabelled tracers to measure cerebral glucose metabolism, blood flow, and neurotransmitter distribution. Focal hypometabolism may underlie epileptogenic zones. During seizures, increased glucose utilization and blood flow are found. We examined the course of alterations in glucose metabolism in patients with intractable partial epilepsy. The most dramatic changes occurred in the inferior temporal regions. Effects up to 48 hr were found after simple partial seizures (SPS) and complex partial seizures (CPS). However, the time course was different for the two types of seizures. The inferior temporal metabolic rate ipsilateral to focus was relatively increased compared with interictal rate in the 24- hr period following a SPS. A nadir occurred in the second 24 hr following a SPS. The rate then rose to an intermediate level after 48 hr. The relative regional increase in ipsilateral metabolism following a CPS persisted for 48 hr before falling. The brain may take longer than 24 hr after a partial seizure to return to its baseline state. Children with partial seizures are followed with serial PET scans to assess the development of hypometabolism in the epileptic focus. Magnetoencephalography (MEG). may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the spatial distribution of epileptiform discharges in cortical depths; MEG may be superior. Digital signal processing is being applied to data from multiple closely spaced electrode arrays. Comparison of invasive localization of epileptic foci using subdural electrodes and noninvasive evaluation is being performed. Seizures in animal models are used to study patterns of neuronal damage and their relation to altered electrophysiology. Somatostatin (SS) neurons are selectively lost in the dentate hilus of patients with longstanding temporal lobe epilepsy. These neurons are vulnerable to non-NMDA but not NMDA-mediated neurotoxicity in cell culture. NBQX, a non-NMDA antagonist, protected against loss of SS as well as neuropeptide Y (NPY)-containing neurons, while MK-801 protected only against the former. Paired-pulse inhibition was lost in both experimental groups. SS and NPY immunoreactive neurons may not be responsible for this type of inhibition. Nitric oxide (NO) synthase inhibitors may increase seizure severity by disrupting cerebral blood flow autoregulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS-02858-03 ERB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuropsychological and Cognitive Studies in Epilepsy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	William D. Gaillard, M.D.	Special Volunteer, CES	ERB	NINDS
	Charles DeCarli, M.D.	Clinical Associate, (CO), CES	ERB	NINDS
	Teresa Blaxton, Ph.D.	Staff Fellow, CES	ERB	NINDS

COOPERATING UNITS *(if any)*
Medical Neurology Branch

LAB/BRANCH
Epilepsy Research Branch, CNP, DIR

SECTION
Clinical Epilepsy Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.8	PROFESSIONAL: 2.6	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects   [ ] (b) Human tissues   [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have performed imaging studies of language organization in normal controls and patients with epilepsy. Using positron emission tomography (PET), activation of cerebral blood flow (CBF) associated with word and object recognition, auditory comprehension, and phoneme, word, and sentence production are localized in the brain. Studies of these functions in normals form the basis for evaluation of the effect of seizure disorders on cognitive processes subserved by temporal lobe and other cerebral structures. In studies of memory, deactivation of CBF during retrieval reflects the effects of earlier encoding. The deactivated regions are those which are engaged in the initial processing of stimuli. During a visual design recognition task, we found deactivation of right primary visual cortex. During an auditory recognition task, there was a relative CBF decrease in bilateral superior and middle temporal regions. We also evaluated a classical learning paradigm using eyeblink conditioning. We found learning specific increases in CBF in regions previously implicated in animal studies, including cerebellum, basal ganglia, frontal cortex, and hippocampus. Data from subdural stimulation, PET, and magnetic resonance imaging (MRI) are integrated using digital image processing techniques. The combined stimulation and PET data allow us to study the relationship between activation and disruption of cognitive activity, and to form more accurate concepts of the organization of cerebral function. These studies will elucidate the function of regions such as the basal temporal language area, which are of clinical importance when surgery for uncontrolled seizures is planned. Digital signal processing techniques are used to confirm anatomic localization of functional mapping. Using surface fitting algorithms, PET, CT, MRI, and subdural electrode positions are aligned. In PET experiments, rest conditions are averaged and subtracted from activated conditions, in order to reveal regions of increased blood flow during task performance. We found a high concordance between PET-CBF and subdural stimulation mapping using a number of different functional tests. This result shows the practicality of noninvasive preoperative functional brain mapping, and also demonstrates the close correlation of disruption and activation studies. We have found significant involvement of the basal temporal language area, which may explain unexpected postoperative deficits, in PET activation studies. During subdural mapping, stimulation of the basal temporal region disrupted implicit memory priming.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02732-08 ERB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacological Studies of Ion Channels in Cultured Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Michael A. Rogawski, M.D., Ph.D	Chief, NES	ERB	NINDS
Others:	S. Donevan, Ph.D.	Vis. Fell. NES,	ERB	NINDS
	S. Subramaniam, M.D. Ph. D.	Vis. Assoc.	ERB	NINDS
	Jong Rho, M.D.	Med.Staff Fell. NES	ERB	NINDS
	T. R. Werkman, Ph.D.	Vis. Fell., NES	ERB	NINDS
	Bjorn E. Svensson, Ph.D.	Spec. Vol., NES	ERB	NINDS
	K. Wayns, B.S.	Lab. Tech., NES	ERB	NINDS*

COOPERATING UNITS *(if any)*

Lilly Research Laboratories

LAB/BRANCH

Epilepsy Research Branch

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 4.32	PROFESSIONAL: 3.52	OTHER: 0.80

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Whole-cell voltage-clamp and single channel recording techniques were used to study drug interactions with N-methyl-D-aspartate (NMDA) and non-NMDA receptor-coupled cation and γ-aminobutyric acid$_A$ (GABA$_A$) receptor-coupled Cl^- channels in cultured hippocampal neurons and with voltage-dependent K^+ channels in fibroblasts transfected with K^+ channel genes. The aim of this work was to explore new strategies for the rational development of antiepileptic drugs based upon their interaction with neuronal ion channel systems. Work was focused in the following areas: (1) studies on the direct activation of GABA$_A$ receptors by barbiturates; (2) evidence for barbiturate-like actions of felbamate (an antiepileptic dicarbamate) and meprobamate (an anxiolytic dicarbamate); (3) studies demonstrating the failure of felbamate to act as an antagonist of the glycine site on NMDA receptors; (4) evidence for an interaction of the anticonvulsant remacemide with the polyamine site on NMDA receptors; (5) effects of the polyamine toxin argiotoxin 636 on NMDA receptors; (6) mechanism of block of the Kv1.2 K^+ channel by aminopyridines and quinine; (7) alaproclate effects on NMDA receptors and the Kv1.2 K^+ channel; (8) K^+ channel activator (cromakalim and diazoxide) effects on anoxia- and 4-aminopyridine-induced hyperexcitability; and (9) neurosteroid potentiation and inhibition of NMDA receptor responses. Felbamate, a newly approve antiepileptic agent, and the related dicarbamate meprobamate, were found to exhibit barbiturate-like effects on GABA$_A$ receptors, both in their activity as modulators of single GABA$_A$ receptor currents and, in the case of meprobamate, to directly activate the receptor. The low toxicity of felbamate could relate, in part, to its failure to produce this latter effect. Studies were continued on the interaction of remacemide (an anticonvulsant drug currently under clinical investigation) with the NMDA receptor. Remacemide was found to allosterically inhibit NMDA receptor channel opening via an action at the NMDA receptor's polyamine site.

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*R. P. Irwin, M.D., CNB, NIMH; C. Hough, Ph.D., BPB, NIMH; D-M. Chuang, PHD., BPB, NIMH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02877-02 ERB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Preclinical Evaluation of Novel Anticonvulsant Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Rogawski, M.D., Ph.D.	Chief, NES	ERB	NINDS
Others:	Shun-ichi Yamaguchi, Ph.D.	Psychologist, NES,	ERB	NINDS
	Sean Donevan, Ph.D.	Visiting Fellow, NES	ERB	NINDS
	Tushar Kokate, Ph.D.	Visiting Fellow, NES	ERB	NINDS
	Bjorn E. Svensson, Ph.D.	Special Volunteer, NES	ERB	NINDS

COOPERATING UNITS *(if any)*

Astra Arcus AB, Södertälje, Sweden

LAB/BRANCH
Epilepsy Research Branch, CNP, DIR

SECTION
Neuronal Excitability Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation of novel antiepileptic drugs in animal seizure models is carried out as a complement to studies on the interaction of these drugs with ion channels in *in vitro* systems. The anticonvulsant activities of a series of 2,3-benzodiazepine noncompetitive non-NMDA (AMPA/kainate) excitatory amino acid antagonist was characterized in the maximal electroshock (MES) test and for protection against kainate-induced seizures. The rank order of potencies of the compounds *in vivo* corresponded with their *in vitro* potencies, supporting the view that the anticonvulsant activity is related to blockade of non-NMDA receptors. Noncompetitive AMPA/kainate antagonists, such as the 2,3- benzodiazepine analogs may offer advantages over competitive antagonists in certain seizure types, especially those associated with high synaptic levels of glutamate. Endogenous metabolites of certain steroid hormones (neurosteroids) can modulate the excitability of central nervous system neurons via direct actions on $GABA_A$ receptors. The ability of a series of isomeric metabolites of progesterone and deoxycorticosterone (3-hydroxy pregnane-20-ones and 3-hydroxy pregnane-21-ol-20-ones) to enhance GABA-evoked chloride currents in cultured hippocampal neurons was compared with their abilities to protect against pentylenetetrazol (PTZ)-induced seizures in mice. Progesterone metabolites with 3-hydroxy in the α-position and 5-H in the α- or β-configuration were highly effective at potentiating GABA-evoked Cl^- current and also showed potent anticonvulsant activity in the PTZ test. The corresponding metabolites with hydroxyl groups in the 3β-position were considerably less potent in enhancing GABA responses and were inactive in the PTZ test. All neurosteroids failed to protect against tonic hindlimb extension in the MES test. At higher doses, neurosteroids effective in the PTZ test also produced motor impairment. Relative motor toxicity was lower (higher protective index) for compounds with the 5α-configuration than for their corresponding 5β-epimers. Certain naturally occurring neurosteroid isomers are highly effective anticonvulsants and this activity is correlated with their ability to potentiate $GABA_A$ receptor responses. Although toxicity (sedation) may be an impediment to the clinical use of neurosteroids in seizure therapy, there is variation among analogs in the extent to which toxicity is produced at anticonvulsant doses suggesting that some neurosteroids could have utility as anticonvulsant agents.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02263-18ETB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical and Pharmacological Studies of Dopamine Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: David R. Sibley, PhD, Chief, MNS/ETB/NINDS
Others: Jean E. Lachowicz, PhD, PRAT Fellow
Tom R. Hollon, PhD, IRTA Fellow
Brian N. Atkinson, PhD, IRTA Fellow
Steven I. Max, PhD, IRTA Fellow
Loyd H. Burgess, PhD, IRTA Fellow
Li-Juan Zhang, PhD, Visiting Fellow
Antonio M. Gonzales, Ph.D., Fogarty Fellow

COOPERATING UNITS *(if any)*
Lab Cell Biol, NIMH; Lab Mammalian Genes/Dev., NICHD; Neurosci. Dept., Chicago Med Sch; Pharm. Dept, Texas Tech U; Psych Dept., Seattle VA Med Ctr; Psych. Dept, Case Western Res. Univ.

LAB/BRANCH
Experimental Therapeutics Branch

SECTION
Molecular Neuropharmacology Section

INSTITUTE AND LOCATION
NINDS,NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 7.75	PROFESSIONAL: 7.35	OTHER: 0.40

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long-term goal of this project is the characterization of neurotransmitter receptor-mediated information transduction, and its regulation, across neuronal membranes. The primary, but not exclusive, model systems under investigation are those for dopamine receptors. In order to characterize dopamine and related receptors at the biochemical and molecular levels and study their regulation, there are two major interrelated lines of research which are ongoing: 1) investigation of the cell biology, function and regulation of the receptors at the protein level; and 2) molecular cloning of the receptor cDNAs/genes and investigation of receptor structure and regulation in normal and pathophysiological states. 1) Cell Biology and Regulation of Dopamine Receptors: Characterization of the functional and regulatory properties of D-1 and D-2 dopamine receptors in various cDNA-transfected cell lines was continued. The D-1A and D-1B receptors were shown to undergo agonist-induced desensitization in CHO cells. The role of cAMP in this response is being tested using mutagenesis techniques. Both short and long isoforms of the D-2 receptor were also shown to undergo agonist-induced desensitization in CHO cells. The D-2S receptor was also down-regulated by agonist treatment whereas the D-2L receptor was up-regulated through a mechanism involving increased receptor synthesis. Both D-2 receptor isoforms were expressed in NG108-15 neuroblastoma cells and shown to couple to K^+ channels, albeit through different G proteins. The D-1A and D-3 receptor proteins were mapped in the CNS using immunohistochemical methods. 2) Molecular Cloning of Dopamine and other Receptors: Both D-1 receptor subtypes were sequenced in the spontaneous hypertensive rat (SHR) which exhibits defective kidney D-1 receptors. No differences in sequence were found in comparison to control rats. Work continued on the cloning of a third "D-1 like" receptor which apparently is linked to the stimulation of phosphatidylinositol turnover and mobilization of calcium. Transgenic "knock-out" mice lacking a functional D-1A receptor were produced and are undergoing characterization. Other transgenic mice lacking the D-1B and D-3 receptors are in production. Chimeric D-2/D-3 and D-2/D-4 dopamine receptors were constructed and expressed for characterization. The 5-HT-6 and 5-HT-7 serotonin receptor subtypes were further characterized with respect to their pharmacology and regulatory properties. A cDNA clone encoding an opiate-like receptor was expressed and characterized.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02826-04 ETB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Regulation of Transmitter Receptor Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Maral Mouradian, M.D.	Chief, Genetic Pharmacology	ETB/NINDS
Others:	S. Yajima, Ph.D.	Visiting Fellow	GPU, ETB
	S-H. Lee, Ph.D.	Visiting Fellow	GPU, ETB
	T. Minowa, Ph.D.	Visiting Fellows	GPU, ETB
	J. Bishop, M.S.	Research Psychologist	GPU, ETB
	M. Minowa, M.S.; D-Q Gao, M.D.; W. Wang, M.D.: Spec. Vol.		GPU, ETB
	P.A. Jose, M.D., Ph.D.	Guest Researcher	GPU, ETB

COOPERATING UNITS *(if any)*
Dept. Physiol., USUHS; Dept. Pediat., G'town Univ. Med. Cent.; Dept. Pharmacol., Creighton Univ., Omaha, NE; LCMN, NIA; Mol. Neurobiol., Dept. Neurol. Univ.Tokyo; Dept. Neurosci., Univ. Cagliari

LAB/BRANCH
Experimental Therapeutics Branch

SECTION
Genetic Pharmacology Unit

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.9	PROFESSIONAL: 3.6	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects [X] (b) Human tissues [X] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main discoveries of the Genetic Pharmacology Unit during FY 94 were the following: (1) Increased intracellular cAMP concentration results in up to 7-fold *trans*-activation of the human <u>D_{1A} dopamine receptor gene promoter</u> in transient transfection assays in SK-N-MC cells. We localized this response to two regions within exon 1 of this gene where nuclear protein interactions were observed. This response appears to be mediated by a novel transcription factor. (2) The *in vivo* function of a 2.3 kb D_{1A} promoter fragment fused to the lacZ gene was tested in transgenic mice. The progeny of many founders were examined but the distribution of staining was unlike that of endogenous D_{1A} receptors. (3) We found a 130 kDa nuclear protein that binds to the negative modulator of the rat <u>D_2 receptor gene</u> in conjunction with Sp1. Current efforts are focused on cloning this protein. (4) *In* vivo analysis of a 353 bp fragment of the rat D_2 gene fused with the lacZ reporter gene in transgenic mice failed to show any blue staining in four lines. (4) We discovered that nuclear extracts from the brains of old rats bind to the promoter region of the D_2 gene much less than those from young rats and may explain the decline in D_2 receptor expression with aging.(5) In the <u>rat BDNF gene</u> which has multiple alternate first exons, we discovered yet another 5' exon. Quantitation of BDNF transcripts having each of these alternate exons revealed that all are most abundant in the hippocampus, intermediate in nigra and cerebellum, and lowest in striatum. However, the magnitude of these differences varied considerably among the alternate first exons suggesting that BDNF gene transcription in the adult brain is regulated by alternate promoters that are differentially active across brain regions. We also found that increased intracellular calcium levels in the BDNF ex-pressing C6 glioma cells results in up to 10-fold increase in the BDNF message, a response which could be blocked by actinomycin D treatment. (6) BDNF function in the adult rat brain was studied using antisense oligonucleotides. Two days after stereotaxic injection of antisense oligos into the striatum, we observed a 3-fold increase in nigral DA content compared with rats receiving nonsense oligos. Seven days later, nigral DA levels returned to normal while striatal levels increased by 2-fold. These observations add support to the hypothesis that BDNF is critical in modulating the function of the nigrostriatal dopamine system in the adult organism. (7) We determined the sequence of an antisense oligodeoxynucleotide that effectively blocks apolipoprotein E message translation in cultured cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02139-20 ETB

PERIOD COVERED
October 1, 1993 through September 31, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Judith R. Walters	Chief, NPS	ETB/NINDS
Others:	Debra Bergstrom	Pharmacologist	ETB/NINDS
	Michael Twery	Senior Staff Fellow	ETB/NINDS
	Kai-Xing Huang	Special Volunteer	ETB/NINDS
	Lisa Thompson	Staff Fellow	ETB/NINDS
	Deborah Kreiss	Staff Fellow	NIGMS

COOPERATING UNITS *(if any)*
Clinical Pharmacology Section, Experimental Therapeutics Branch

LAB/BRANCH
Experimental Therapeutics Branch, CNP

SECTION
Neurophysiological Pharmacology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 7.0	PROFESSIONAL: 6.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) The potencies of a series of 11 highly efficacious dopamine (DA) agonists for *in vivo* inhibition of DA single cell firing correlate better with *in vitro* binding affinities at D3 than at D2 receptors in CHO cells transfected with cDNAs encoding for D3 and D2L receptors, respectively. These results support a functional contribution of the D3 receptor subtype in the autoreceptor-mediated regulation of DA cell activity. However, infusion of DA D2 and D3 receptor antisense oligonucleotides into rat substantia nigra has, to date, produced no selective modifications of firing which can be distinguished from nonselective effects.

(2) In models of basal ganglia organization, DA receptor stimulation is thought to indirectly decrease neural activity in the subthalamic nucleus due to disinhibition of the globus pallidus. However, systemic administration of the nonselective DA agonist apomorphine has been found to double the average firing rate of subthalamic neurons. Two D1 agonists also increased the firing rate of subthalamic neurons, a D2/D3 agonist produced smaller effects. Local infusion of D1 agonists also stimulated pallidal activity, indicating a potential excitatory role of DA locally in the subthalamic nucleus. Investigation of possible sites of action of D1 and D2 agonists in 6-OHDA-lesioned rats through local infusions has demonstrated short-term plasticity and indicates effects vary depending on whether D1 receptors and D2/D3 are stimulated simultaneously or 5 to 10 min apart.

(3) Cannabinoid receptor distribution suggests that cannabinoids may influence basal ganglia motor function by actions on the striatal output pathways. The finding that cannabinoid agonists attenuate DA agonist-induced rotation in rats with 6-OHDA-induced DA cell lesions indicate that cannabinoid receptor stimulation influences both D1- and D2-mediated processes, although D1 processes appear to be affected to a greater degree.

(4) We have found a high level of AP-1 binding in the rat striatum which is enhanced after DA depletion by reserpine treatment or by 6-OHDA-induced DA cell lesion. Binding was further enhanced by combinations of D1 and D2 DA agonists in the 6-OHDA-lesioned rats but only by D1 agonists in normal rats. The observations indicate that AP-1-mediated changes in gene expression may contribute to alterations in agonist sensitivity in the striatum after DA cell lesion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02265-18 ETB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology, Biochemistry and Physiology of Central Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thomas N. Chase, M.D. Chief ETB/NINDS

Others: Jeff Anderson, PhD, IRTA Fellow
Robert Boldry, PhD, IRTA Fellow
Stella Papa, M.D., Visiting Fellow
Leo Verhagen-Metman, M.D.Visiting Associate
Italo Linfante, M.D., Visiting Associate
Pierre Blanchet, M.D.,Special Volunteer
Eduardo Locatelli, M.D., IRTA

COOPERATING UNITS (if any)

Georgetown Univ.; Hosp De La Salpetriere, Paris; NIMH; NIDCD; NIA; NIDR; Univ. Pavia, Italy; Royal Ottawa Hosp., Canada, University of Toronto

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Clinical Pharmacology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 9.64	PROFESSIONAL: 7.64	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[X] (a1) Minors
[X] (a2) Interviews

[X] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. Altered motor responses complicating L-Dopa therapy of Parkinson's disease initially arise as a consequence of the loss of striatal dopamine storage due to dopaminergic terminal degeneration, but later secondarily reflect postjunctional alterations. In Parkinsonian rats, postsynaptic changes, leading to increased responsivity of D2 dopamine receptor-mediated striatal efferents and diminished responsivity of D1 mediated projections, occur with intermittent, but not continuous, dopaminomimetic administration. Since striatal D2 receptor binding remains essentially unchanged and there is only mild up-regulation of D1 receptors, alterations in dopaminoceptive peptidergic and downstream glutamatergic systems are presumably responsible. Blockade of the NMDA subtype of glutamate receptors exerted differential effects on dopamine agonist-induced rotational behavior that depend on which dopamine receptor subtype is activated and the previous exposure to dopamine agonists. NMDA antagonists might thus be expected to influence dopaminomimetic responses clinically and counter certain of the motor complications associated with chronic L-Dopa treatment.

2. Earlier studies suggested that drugs acting to extend the biologic half-life of L-Dopa and dopamine will confer prophylactic as well as palliative benefit to Parkinsonian patients. Now we find that coadministration of a novel inhibitor of catechol-O-methyltransferase substantially prolongs the response to L-Dopa/carbidopa without significantly affecting the type or severity of adverse effects. The addition of talcapone to the therapeutic regimen of Parkinsonian patients should thus prove useful in controlling wearing-off fluctuations and other motor response complications.

3. The glycine prodrug, milacemide, which positively modulates NMDA receptor-mediated glutamatergic transmission, transiently increased overall symptom severity in Parkinsonian patients, lending further support to our view that pharmaceuticals that block certain glutamate receptor subtypes may assist in the treatment of this disease. The selective kappa receptor agonist, spiradoline, given to evaluate the clinical effects of the enhanced dynorphinergic transmission attending chronic levodopa administration to parkinsonian rats, produced dose limiting adverse effects that precluded attainment of dose levels approximating those affecting rodent motor performance.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02667-10 MNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Analysis of Involuntary Movements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Mark Hallett, M.D.	Chief	HMCS	MNB	DIR	NINDS
Others:	Camilo Toro, M.D.	Visiting Scientist	HMCS	MNB	DIR	NINDS
	Barbara Karp, M.D.	Chief, Consultation Service		OCD	DIR	NINDS
	Stephen Grill, M.D., Ph.D.	Clinical Associate	HMCS	MNB	DIR	NINDS
	Joyce Grisson, M.D.	Special Volunteer	HMCS	MNB	DIR	NINDS
	Ali Samii, M.D.	Visiting Associate	HMCS	MNB	DIR	NINDS
	Mary K. Floeter, M.D.	Senior Staff Fellow		OCD	DIR	NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.5	PROFESSIONAL: 2.9	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Involuntary movements have often been difficult to classify clinically. Clinical and physiologic analysis of a continuing series of patients has led to new classifications and pathophysiologic insights. Patients with myoclonus have been studied to seek further understanding of this confusing involuntary movement. Detailed studies are in progress on the opsoclonus-myoclonus syndrome and on the phenomenon of negative myoclonus. Analysis is ongoing of the physiology of periodic movement in sleep.

Extensive clinical and physiologic studies have continued in patients with palatal tremor (myoclonus). We have further data confirming the division of these patients into two groups, essential and symptomatic.

A study of movement-related cortical potentials in patients with dystonia (hand cramps) have revealed an abnormality of cortical activation. This has been confirmed in additional studies with event-related desynchronization of the EEG and somatosensory evoked potentials.

We have studied 5 patients with stiffman syndrome in attempts to characterize the spinal and supra-spinal mechanisms responsible for the generation of symptoms. Abnormalities of reflex mechanisms including lack of vibratory inhibition of H-reflex and abnormalities of reciprocal inhibition of the H-reflex were found in all patients indicating a dysfunction of normal inhibitory mechanisms involved in muscle relaxation.

The movement related cortical potentials accompanying tics have shown a slow rising negativity similar to a Bereitschaftspotential before voluntary movement in some patients.

We have initiated studies of inhibitory reflexes in patients with familial hyperekplexia.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02669-10 MNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Analysis of Voluntary Movement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Mark Hallett, Chief, HMCS, MNB, DIR, NINDS

Others: C. Toro, M.D., Vis. Sci., HMCS, MNB
A. Samii, M.D., Vis. Assoc., HMCS, MNB
M. Deiber, M.D., Ph.D., G. Resear, HMCS, MNB
V. Ibanez, M.D., Ph.D., Vis. Assoc.
M. Demirci, M.D., Ph.D., Spec. Vol., HMCS, MNB
N. Sadato, M.D., Vis. Fell., HMCS, MNB
B. Mercuri, M.D., Vis. Fell., HMCS, MNB
M. Keidel, M.D., Spec. Vol., HMCS, MNB
M. Leonardo, Spec. Vol., HMCS, MNB
S. Grill, M.D., Ph.D., Sr. Clin. Assoc., HMCS, MNB
S. Massaquoi, M.D., Sr. Clin. Assoc., HMCS, MNB
E. Wassermann, M.D., Clin. Invest., OCD, NINDS

COOPERATING UNITS *(if any)*

Department of Rehabilitation Medicine, Clinical Center
Department of Nuclear Medicine, Clinical Center

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 9.4	PROFESSIONAL: 8.7	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[X] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of the cerebellar contribution to multijoint coordination focussed on planar 2-joint arm movements. Mathematical modeling preliminarily suggests that the cerebellum controls force transitions by linearly processing velocity feedback signals from both joints. A second issue is the role of the cerebellum in kinesthesia, the sense of movement. Results show a deficit in appreciation of velocity and duration in patients with cerebellar deficits. Other studies have focused on the control of balance and gait. Patients with cerebellar disorders and progressive supranuclear palsy are being studied.

Using O-15 labelled water as a marker for cerebral blood flow in positron emission tomography (PET) studies, we have been working on methods for improved anatomic correlation of regions of metabolic change by superimposing the PET image onto an MRI image. In studies of PET and functional MRI we have shown plasticity of the motor cortex with transient limb deafferentation produced by ischemic block and intermodal plasticity in the early blind. We have also shown frequency-dependent, differential activation of sensorimotor cortical areas using repetitive movements and repetitive peripheral nerve stimulation. We have also demonstrated activation of cortical regions including primary motor cortex with imagination of movement.

Studies of somatosensory evoked potentials, movement related cortical potentials (MRCP), event related desynchronization, EEG coherence have been integrated with results from PET and functional MRI scanning. These techniques now provide complementary topographic and timing details of pre- and intramovement brain activity. A model of MRCP sources has been validated though comparison with areas of PET activation and subdural electrode potentials. Studies of changes in scalp potentials and cortical oscillations with voluntary muscle relaxation are ongoing. Studies are ongoing to record muscle spindle and cutaneous mechanoreceptor activity during voluntary movements and passive stretch. Results suggest that both are adequate in providing signals to the nervous system related to movement velocity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02711-09 MNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Utility and Physiology of Botulinum Toxin for Involuntary Movement Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Mark Hallett, M.D.,	Chief	HMCS	MNB	DIR	NINDS
Others:	Barbara I. Karp, M.D.	Chief, Consultation Service		OCD	DIR	NINDS
	Ali Samii, M.D.	Visiting Associate	HMCS	MNB	DIR	NINDS

COOPERATING UNITS *(if any)*

Speech Pathology Unit, NIDCD

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying the efficacy of local injections of botulinum toxin for the treatment of different types of focal dystonias. Botulinum toxin injected in small doses directly into muscle, binds to and inactivates the neuromuscular junction. Dystonic contraction is decreased and function improves Treatment is well-tolerated with minimal side effects. We have also used botulinum toxin to study the physiology of focal dystonias. A double-blind trial of botulinum toxin for hand dystonia (e.g. writer's cramp and musician's cramp) showed a significant improvement in function following injection of active botulinum toxin compared to placebo. Open-label studies have shown that 84% of patients have at least short-term benefit. Longer follow-up shows that approximately 50% of patients have persistent benefit. Women with nonlocalized symptoms or dystonic cramp who require less frequent injections were especially likely to continue treatment. Botulinum toxin injection is well-tolerated. Botulinum toxin injections have been similarly effective in our patients with other types of dystonia. Twelve patients with tremor have been treated to date. All of the patients with torticollis and head tremor who received at least 3 injections had at least mild improvement. 50% of the patients with arm tremor had substantial improvement. One patient noted increased tremor after injection. We are conducting a phase I-II trial of botulinum toxin type F to see if this will benefit patients who have lost response to type A. Type F appears to have similar efficacy and side effects to type A, although the duration of action is slightly less.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02712-09 MNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Noninvasive Stimulation of Human Central Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Mark Hallett, M.D., Chief, HMCS, MNB, DIR, NINDS

Others:

Eric Wassermann, M.D.	Clinical Investigator			OCD	NINDS
Ali Samii, M.D.	Visiting Associate	HMCS	MNB	DIR	NINDS
Joyce Grissom, M.D.	Special Volunteer	HMCS	MNB	DIR	NINDS
Katsunori Ikoma, M.D.	Special Volunteer	HMCS	MNB	DIR	NINDS
Bruno Mercuri, M.D.	Visiting Fellow	HMCS	MNB	DIR	NINDS

COOPERATING UNITS *(if any)*
Speech and Voice Pathology Unit, NIDCD

LAB/BRANCH
Medical Neurology Branch, CNP, DIR

SECTION
Human Motor Control Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.8	PROFESSIONAL: 2.5	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are using new methods for the noninvasive stimulation of the human cortex. Stimulation can be with a high-voltage electrical pulse or with magnetic stimulation. One purpose is to use these methods for noninvasive localization of different parts of the human cortex including motor cortex, sensory cortex and language cortex. Another purpose is to study cortical and corticospinal physiology in normal humans and in different disease states. We have continued to improve our techniques of topographical mapping with TMS. We are investigating CNS plasticity following lesions, finding new phenomena that have implications for theories of the organization of the motor system and mechanisms of reorganization.

Exercise produces competing effects on corticospinal excitability. These effects are present in differing proportions in disorders producing fatigue. This phenomenon may yield an unambiguous marker for organic disease in patients complaining of fatigue. Motor evoked potential amplitudes increase prior to movement. Our experiments are yielding answers about how and where this change takes place. This is important in explaining how motor commands are generated and transmitted.

Motor maps enlarge with acquisition of skills and motor learning. This process correlates closely with cognitive aspects of skill acquisition and learning, demonstrating the role of the primary motor cortex in the process.

The physiology of the supplementary motor area (SMA) in humans is largely unexplored and its role in movement is controversial. Anatomical data suggest direct links with anterior horn cells. Our studies suggest a role both in the modulation of segmental reflexes and control of sequential movements.

The pathophysiology of tics is mysterious. They are voluntary phenomena triggered by a pathological process. Our data suggest a means of altering this pathological desire to move.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02792-06 MNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuropsychological Investigations of Human Cognition and Mood State

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Grafman, Ph.D., Chief, CNS, MNB, NINDS
Others: M. Stark, Ph.D., IRTA Fellow, CNS, MNB, NINDS, V Goel, Ph.D., Vis. Fellow, CNS, MNB, NINDS; P. Nichelli, M.D., Vis. Scientist, CNS, MNB, NINDS; L. Rueckert, Ph.D., IRTA Fellow, CNS, MNB, NINDS; I. Appollonio, M.D., Spec. Vol., CNS, MNB, NINDS; A. Partiot, M.D., Spec. Vol., CNS, MNB, NINDSM. Hallett, M.D., Chief, MNB, NINDS; A. Sirigu, Ph.D., B. Dubois, INSERM U. 289, Hop. Salpetriere, Paris; F. Boller, M. D., Ph.D., INSERM U. 324, Cent. Paul Broca, Paris, France; S. Rao, Ph.D. Med. Coll. Wisc., Milwaukee, WI; K.Holyoak, Ph.D., Dept. Psychol. UCLA, Los Angeles, CA (continued below)

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medical Neurology Branch, CNP, DIR

SECTION
Cognitive Neuroscience Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 14.1	PROFESSIONAL: 14.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Current studies in the Cognitive Neuroscience Section focus on planning, thinking, and reasoning; memory and amnesia; visual attention, spatial perception, and object recognition; and emotion/social cognition. Both single-case and group design studies are used. Normal controls, inpatients, and outpatients with central nervous system impairment are recruited for studies. Planning, thinking and reasoning are studied in experiments focusing on schema development, generation of cognitive plans, analogical thinking, script event generation and verification, number processing and calculation, knowledge representation, and divided resources Memory and amnesia are studied in experiments focusing on implicit and explicit encoding and retrieval, priming, autobiographic recall, discourse processing, naming and word retrieval, and categorization tasks. Visual attention, spatial perception, and object recognition are studied in experiments focusing on spatial frequency, contrast sensitivity, object knowledge and feature verification, visual-spatial localization, spatial, selective, and sustained attention, and local-global properties of stimuli. Emotion and social cognition are studied in conjunction with cognitive experiments examining attention and memory, rule retrieval, and inhibition. The development of theoretically valid and testable models of cognitive processing is a primary aim of the Section. We study patients with focal and degenerative lesions in order to topographically map components of cognitive processing to brain regions and systems. Pharmacologic challenge and infusion studies are done to evaluate the dissociability of hypothesized components of cognitive processing. Transcranial magnetic stimulation, functional magnetic resonance imaging, positron emission tomography, and event-related brain potentials are all employed to examine the topographic location and computational properties of cognitive components.

J. Hendler, Ph.D., Dept. Comp. Sci., U. Md, College Park, MD; L. Spector, Ph.D., Dept. Comm. & Cogn.Sci., Hampshire Coll., Amherst, MA; C. Junque, Ph.D., Dept. Psychobiol., Univ. Barcelona, Spain; A. Salazar, M.D., Dept. Neurol., WRAMC, Washington, D.C.; B. Fantie, Ph.D , Dept Psychol., Am. Univ., Washington, D.C.;K. Epstein, Ph.D , Dept. Math., Gallaudet Univ., Washington, D.C.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02909-01 MNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

The use of Modern Neuroscience Techniques to Perform Brain-Behavior Mapping

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Jordan Grafman, Ph.D.	Chief, CNS, MNB, NINDS
Others:	P. Nichelli, M.D.	Visiting Scientist, CNS, MNB, NINDS
	L. Rueckert, Ph.D.	IRTA Fellow, CNS, MNB, NINDS
	A. Partiot, M.D.,, Ph.D.	Special Volunteer, CNS, MNB, NINDS
	I. Appollonio, M.D.	Special Volunteer, CNS, MNB, NINDS
	V. Goel, Ph. D.	Visiting Fellow, CNS, MNB, NINDS
	B. Dubois, M.D.	INSERM U. 289 Hopital Salpetriere, Paris, France
	*	

COOPERATING UNITS (*if any*)

Walter Reed Army Medical Center, Wash, DC; National Naval Medical Center, Bethesda, MD; Centre Paul Broca, Paris, France; Hopital Salpetriere, Paris, France; Hospital Clinicas, Montevideo, Uruguay

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Cognitive Neuroscience Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.1	PROFESSIONAL: 2.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Current neuroscience techniques used in the Cognitive Neuroscience Section include positron emission tomography (PET), functional magnetic resonance imaging (fMRI), rapid-rate transcranial magnetic imaging (rTMS), event-related brain potentials (ERPs), and pharmacologic challenges (PC). Primarily within-group designs are used although both fMRI and rTMS can be used with single cases and our research is proceeding in this direction. Our PET program is focusing on developing methods to reliably activate various locations within the human prefrontal cortex. In this regard, we are using tasks that require subjects to plan, to develop thematic knowledge, to put themselves in someone elses point-of-view and to mentally execute a set of activities. The fMRI technique is currently being used to study basic cognitive processes such as word production, mental calculation, and selective attention. rTMS is being used to map cortical functions during activity and learning, to interfere with ongoing cognitive processing, and to facilitate cognitive processing. The ERP recordings are being used in order to better identify the components of verbal and nonverbal working memory in normal subjects and in clinical populations. We have judiciously used PC in order to order examine the effects of anticholinergic medication on autobiographical memory and selective attention.

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*Continued:

M. Hallett, M.D.	Chief, MNB, NINDS
N. Sadato, M.D.	HMCS, MNB, NINDS
A. Pascual-Leone, M.D.	HMCS, MNB, NINDS
P. Pietrini, M.D.	LNS, NIA
E. Wasserman, M.D.	HMCS, MNB, NINDS
N. Lange, Ph.D.	BRS, NINDS
P. Jezzard, Ph.D.	LCE, NHLBI
R. Turner, Ph.D.	National Hospital, Queen Square, London
D. Ruchkin, Ph.D.	Dept. of Physiology, Univ. of Maryland

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02038-22 MNB

PERIOD COVERED

October 1,1993 through September 30,1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Combined Clinical, Viral and Immunological Studies of Neuromuscular Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.C. Dalakas, M.D., Chief, NDS, MNB, DIR, NINDS

Others:

Edward Cupler, M.D., Neurologist, NDS, MNB,DIR, NINDS
Elizabeth Sekul, M.D., Neurologist, NDS, MNB, DIR, NINDS
M. Monzon, Ph.D.,Special Volunteer, NDS,MNB,DIR,NINDS
I. Illa, M.D., Exchange Scientist, NDS,MNB, DIR,NINDS
B. Sonies, Ph.D., Speech Pathologist, CC, DIR, NINDS
M. Ropka, M.D., Director, IP, NR
Lev Goldfarb, M.D., Director, DNA Sequencing, DIR, NINDS
M. Agboatwalla, M.D.,Child Specialist, Karachi, Pakistan
A. McLaughlin, Ph.D., DRRP,OD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Neuromuscular Diseases

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.4	4.0	2.4

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[X] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) of chronic diseases of the neuromuscular system and design effective therapies. Current studies involve patients with polymyositis/dermatomyositis, post-polio syndrome, amyotrophic lateral sclerosis (ALS), demyelinating polyneuropathies, neuromuscular diseases associated with HIV infection, hypokalemic periodic paralysis and Duchenne muscular dystrophy. The pathogenesis of post-polio syndrome is explored with a series of electrophysiological, virological, immunological and histological studies. The findings are compared with those seen in patients with acute paralytic poliomyelitis and other motor neuron diseases. Persistent or mutant poliovirus is sought in these patients' tissues using tissue cultures, PCR, and in situ hybridization. Because abnormal immunoregulation was found in some patients, a double-blind placebo-controlled trial using prednisone was conducted. The mechanism of post-polio fatigue, a common and disabling symptom in many patients, is under study using magnetic resonance spectroscopy. Sequence of the β amyloid precursor protein gene is performed in patients with familial and sporadic inclusion body myositis. The spectrum of neuromuscular disorders associated with HIV infection has been studied and the role of the virus in the cause of neuropathy or myopathy is investigated with a variety of immunocytochemical studies, *in situ* hybridization and PCR. The antiretroviral drug AZT was found to cause an unique myopathy characterized by abnormal mitochondria as determined by various morphological, molecular, biochemical and immunocytochemical studies. A longitudinal study of HIV-positive patients that develop myopathic symptoms while on AZT is conducted with serial muscle biopsies to assess factors associated with the development of myopathy. Patients with AZT-myopathy were found to have low muscle carnitine level. This has prompted carrying out an ongoing randomized controlled clinical trial using oral L- carnitine. Randomized-controlled clinical trials are conducted with high-dose intravenous immunoglobulin in patients with polymyositis/dermatomyositis, chronic inflammatory and paraproteinemic demyelinating polyneuropathies, amyotrophic lateral sclerosis and Duchenne muscular dystrophy. A controlled study using dichlorophenamide, a carbonic anhydrase inhibitor, is also conducted in patients with hypokalemic periodic paralysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02531-13 MNB

PERIOD COVERED
October I, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies in Neuromuscular and CNS Diseases and Their Experimental Models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.C. Dalakas, M.D.	Chief, NDS	MNB, DIR, NINDS
OTHERS:	M. Monzon, Ph.D.	Special Volunteer, NDS	MNB, DIR, NINDS
	I. Illa, M.D., Ph.D.	Exchange Scientist, NDS	MNB, DIR, NINDS
	E. Cupler, M.D.	Clinical Associate, NDS	MNB, DIR, NINDS
	C. Semino-Mora, M.D.	Special Volunteer, NDS	MNB, DIR, NINDS
	N.D. Epstein, M.D.	Molecular Biologist	CHB, DIR, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medical Neurology Branch, CNP, DIR

SECTION
Neuromuscular Diseases

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.85	PROFESSIONAL: 3.5	OTHER: 1.35

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects [X] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Section runs the Laboratory of Muscle Enzyme Histochemistry that processes up to 300 muscle and nerve biopsies per year for diagnostic and research studies. Examined muscles are from patients with: neuromuscular manifestations related to systemic, autoimmune, viral, metabolic, endocrine or infectious diseases; primary neuromuscular disorders, such as polymyositis, dermatomyositis, neurogenic muscular atrophies, muscular dystrophies, post-polio syndrome, polyneuropathies, mitochondrial encephalomyopathies; and patients with biochemical and genetic muscle diseases, such as central core disease or hypertrophic cardiomyopathy. The Laboratory is also involved in the following immunological, biochemical and virological studies that examine the susceptibility of the muscle and nerve to immune or viral mediated injuries: (a) Study the regeneration of human muscle in health and disease and the maturation of satellite cells by examining the expression of neural cell adhesion molecules and laminins; (b) study the susceptibility of muscle and nerve to infection with retroviruses and the ability of HIV and HTLV-I or HIV and HTLV-I-infected lymphoid cells to infect human myotubes in culture and induce expression of MHC-antigens; (c) study the expression of the poliovirus receptor in human muscle *in vivo* and *in vitro*, and the ability of the poliovirus to infect and replicate in human myotubes; (d) study the effect of cytokines and lymphokines on human myotubes and examine *in vitro* if potentially therapeutic agents such as IVIg can inhibit their toxic or immunopotentiating effect; (e) examine the role of ICAM-I in enhancing myocytotoxicity *in vivo* and *in vitro* by promoting the adhesion of cytotoxic T cells to myotubes; (f) study the toxicity of AZT to muscle mitochondria, mitochondrial oxidative phosphorylation and mitochondrial DNA by applying AZT to human muscle in culture; (g) study the effect of L-carnitine in reversing the mitochondrial abnormalities induced by AZT on human myotubes *in vitro*; and (h) using animal models. These models will be employed to study: (1) the pathogenesis of retrovirus-induced inflammatory myopathy by examining muscles from monkeys infected with the simian immunodeficiency virus; (2) the mechanism of AZT-induced mitochondrial myopathy by examining the structural, metabolic and functional alterations in the muscle mitochondria of healthy rats injected with AZT; (3) the effect of L-carnitine in reversing or improving the AZT-induced myopathy in the rats; and (4) the mechanism by which dideoxycytidine induces neurotoxicity in healthy rats.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02240-18 NEB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiology of Dementia and Other Neurodegenerative Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Irene Litvan, M.D.	Senior Staff Fellow	NEB, DIR, NINDS
Others:	Karin B. Nelson, M.D.	Acting Chief	NEB,DIR,NINDS
	Luis D'Olhaberriague, M.D.	Guest Researcher	NEB,DIR,NINDS
	Darsy Calderon, M.P.H.	Special Volunteer	NEB,DIR,NINDS

COOPERATING UNITS *(if any)*
Carlos A. Mangone, M.D.,Francisco Santojanni General Hospital, Buenos Aires, Argentina, SA

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.12	PROFESSIONAL: 2.12	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Neuroepidemiology Branch and an international group of expert neuropathologists have developed neuropathologic criteria for diagnosis of progressive supranuclear palsy (PSP). The objective of the PSP study is to determine the neuropathologic spectrum of PSP and better distinguish it from other disorders. The reliability of neuropathologists in using these neuropathological criteria was tested. Prospective studies are underway to validate previously clinically proposed criteria and to develop a model of clinical diagnosis PSP and related disorders, using clinical material confirmed by postmortem examination.

Vascular dementia is the second most common cause of dementia in the elderly. Analytic studies to determine risk factors for <u>vascular dementia</u> (VAD) and dementia of Alzheimer type(DAT) are being planned for performance in Argentina in a well-defined population. Although diagnostic accuracy is important in epidemiologic studies, most case-control studies on dementia do not have well-defined populations. Neuroradiological anatomic (MRI) and perfusion imaging (Tc99 HMPAO cerebral SPECT) studies useful to differentiate DAT from VAD will be used in our studies.

In line with our focus to identify the pathogenesis of neurodegenerative disorders, we plan collaborative studies with the epidemiology group of the Nationakl Institute on Aging, to evaluate the role of documented head injury in the later development of DAT by the examination of World War II veterans.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02307-18 NEB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Educational Resources in Neurological Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Karin B. Nelson, M.D.	Acting Chief	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.02	PROFESSIONAL: 0.02	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because there is a severe shortage of available manpower in neuroepidemiology, the Neuroepidemiology Branch (NEB) has developed an active teaching program for current and future collaborative investigators. Particular attention has been given to junior members of the American Academy of Neurology (Neurology residents). The NEB has participated actively in the annual courses of the American Academy of Neurology, in an effort to increase the interest in neuroepidemiology. The following are some of these activities:

(1) Full-day course final lecture participation, American Academy of Neurology: *"Clinical Research Methods", Washington, D.C.*

(2) World Federation of Neurology, Research Group on Neuroepidemiology Annual Meeting, Bethesda, M.D.

(3) Organization of a symposium on Neurotoxicology, to be presented at the meeting of the International Child Neurology Association, to be held conjointly with the Child Neurology Society in early October, 1994.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02370-16 NEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Racial and Geographic Differences in Occurrence of Neurologic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Karin B. Nelson, M.D.	Acting Chief	NEB, DIR, NINDS
Others:	Danyang Chen, M.D.	Visiting Associate	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.62	PROFESSIONAL: 0.62	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[X] (a1) Minors

[X] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of these studies is to accurately document demographic environmental and geographic differentials in the prevalence of major neurologic disorders by survey of geographically defined populations and cerebrovascular disease.

Using information from the two National Health and Nutrition Examination Surveys (NHANES I and II), probability sample surveys of the US population, detailed information was utilized concerning self-reported sleep disorders, neck pain, and other neurologic problems. Overall, 39% of Americans reported sleep problems. These were more frequent in women and increased with advancing age,-poor health, depression, and chronic disease. Neck pain lasting at least two weeks was reported by 8.2% of surveyed Americans, and was more common in women; 5% of those with neck pain had had surgery for this problem. The prevalence of neck pain increased with age until a plateau in those aged in the 60s. Both sleep difficulties and neck pain occurred with substantial frequency in this country. Of the more than 10,000 respondents, 9.8% reported migraine, 0.49% persons reported Parkinson's disease, 0.22% multiple sclerosis, 0.69% epilepsy, 2.3% transient ischemic attacks, and 1.3% strokes.

In 1993, the World Health Organization requested the participation of Dr. Gustavo C. Roman, then Chief, NEB, in a team of scientist which traveled to Cuba to investigate an outbreak of optic and peripheral neuropathy in the island. As of January 1994, more than 50,000 cases were reported making this one of the largest epidemics of neurologic disease on record

This project has been completed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02746-08 NEB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Phenobarbital Clinical Trial in Children with Febrile Seizures*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS
Others:	Deborah Hirtz, M.D.	Pediatric Neurologist	DNB, DCDND, NINDS
	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS

COOPERATING UNITS *(if any)*
Jacqueline Farewell, M.D , Dept. of Neurosurgery, Univ. of Washington, Seattle, WA

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects   [ ] (b) Human tissues   [ ] (c) Neither
  [x] (a1) Minors
  [ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of the study are to assess the effects of phenobarbital, a commonly prescribed anti-convulsant, on tests of intelligence and behavior in children. The design of this study permitted comparison of measures of tested intelligence and of behavior in children with febrile seizures who had been treated with phenobarbital, and in a group of seizure-free control children. A comparison of the groups allowed assessment of benefit and risk of treatment for a common childhood neurologic problem.

*[This study supports the DNB/ND/NINDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Deborah G. Hirtz, DNB, DCDND, NINDS, and the contractor of the study is the University of Washington.]

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02838-04 NEB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Retroviral Diseases of the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

co-P.I.:	Karin B. Nelson, M.D.	Acting Chief	NEB, DIR, NINDS.
	Clarence J. Gibbs Jr, Ph.D.	Deputy Chief	CNNS, DIR, NINDS
Others:	Aurora K. Pajeau, M.D.	Clinical Associate	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*
Clarence J. Gibbs Jr, Ph.D., DIR, CNSS, NINDS; William A. Blattner, M.D., C. DCE, EEB, NCI.

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.72	PROFESSIONAL: 0.72	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A door-to-door field survey of the prevalence of clinically diagnosed tropical spastic paraparesis (TSP) in St. Catherine Parish, Jamaica, was carried out by Dr. Pajeau in collaboration with the Statistical Institute of Jamaica and the University of the West Indies, Kingston. Serum samples were analyzed at the University of the West Indies for HTLV-I, B-12, folate and treponemal antibodies. Subjects were referred to the University of the West Indies TSP Clinic for follow-up in Phase III of the study. Data are being analyzed for clinical diagnosis, seropositivity, and for other factors in the differential diagnosis. A nested case control study is planned to determine risk factors for development of TSP.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02861-03 NEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Guillain-Barré Syndrome

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

P.I.:	Gustavo C. Roman, M.D.	Chief	NEB, DIR, NINDS

COOPERATING UNITS (*if any*)

Pan American Health Organization (PAHO); Peking Union Medical College (PUMC), Beijing China

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
  [X] (a1) Minors
  [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This study will determine the incidence of Guillain-Barré syndrome in Latin America, as part of the Pan American Health Organization 's program for poliomyelitis surveillance. Studies are also in the planning stages in the People's Republic of China.

This project was completed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02862-03 NEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurocysticercosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Gustavo C. Roman, M.D.	Chief	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

Julio Sotelo, M.D., Research Division, F. G. Pedroza, M.D. Neuroepidemiologia, Mex. Natl. Inst. Neurol Neurosurg; M. Cruz, M.D. Ecuadorean Acad. Neurosci., Quito, Ecuador

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects   [ ] (b) Human tissues   [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Neuroepidemiology Branch, is conducting a study to define the prevalence of neurocysticercosis (NCC) in populations hyperendemic for taeniasis. As seizures and headaches have been correlated with NCC, the NEB is studying the prevalence of epilepsy and headache in Naulinco, Veracruz, Mexico, to determine the frequency of NCC. This study will determine the natural history of NCC in endemic regions of Mexico and Ecuador.

This project has been completed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02863-03 NEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The California Cerebral Palsy Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

Dr. Judith Grether; Dr. Susan Cummins; Birth Defects Monitoring Group, Department of Health Services, California; Health Officers Association of California

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[x] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has established a population-based registry of children with cerebral palsy (CP) among births 1983-1985 in four San Francisco Bay Area counties. Elements completed include: (a) Study of demographic and medical characteristics related to the occurrence of cerebral palsy in a contemporary American population; (b) CP in twins. Twin pregnancies produced a child with CP twelve times more often than singleton pregnancies. Much but not all of this risk was related to the tendency of twins to be low in birthweight. Twins of unlike-sex pairs, necessarily dizygotic, were not at lower risk than twins of like sex pairs. If one twin died *in utero*, the surviving co-twin was more than 100 times more likely than a singleton to have CP. Twinning is increasing in developed countries, and is likely to contribute more children with CP. Paper published, a confirmation in another country completed and published and an extension in a population that permits evaluation of other neurologic outcomes (the NCPP) completed and submitted for publication; (c) a case control- study within the California population-based cohort was undertaken to examine whether in utero exposure to magnesium sulfate was associated with diminished risk of CP in children of birthweight <1500g. It was the finding is dramatic and potentially important: infants of women treated with magnesium had a substantially lower frequency of CP. This study, including careful scrutiny for potential confounding factors, has been completed and submitted for publication. In progress; (d) an International Collaborative Study of Childhood Neurologic Disorders in Multiple Births has been initiated and a colleague from Western Australia will spend 6 months in the NEB to work on these data, beginning December 1994; (f) Low birthweight is an important risk factor for CP. A study of risk factors for CP in infants <1500 g, comparing survivors with CP with children of that birthweight who survived without CP is underway.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02866-03 NEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Reliability of Diagnoses of First Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joseph M. Scheller, M.D.	Special Expert	NEB, DIR, NINDS
Others:	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

Steven Weinstein, M.D., Neurology Department, Children's Hospital National Medical Center.
James Chamberlain, M.D. Neurology Department and Emergency Medical Trauma Center, CHNMC

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
  [x] (a1) Minors
  [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We examined the consistency of diagnosis of a first seizure in children seeking care at a multispecialty urban teaching hospital, investigating whether the episode described was a first seizure, a nonfebrile seizure, whether it was symptomatic of an underlying illness, and how that seizure should best be descriptively classified. Among other information sought were the source of the medical history, training of person in medical facility who recorded the history, length of time from episode to recording of history. At least two versions of the history were recorded, and a sample audiorecorded; versions of the diagnostic impressions were compared for consistency and for patterns of any differences observed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02867 -03 NEB

PERIOD COVERED

October 1, 1993 through September 30 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurologic Morbidity and Its Antecedents Within the NCPP Dataset

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Co-P.I.:	Jonas Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS
	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

BFSB, NINDS

LAB/BRANCH

SECTION

Neuroepidemiology Branch,

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	0.0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Collaborative Perinatal Project of the NINDS data set continues to be an important resource for information relating maternal and pregnancy and perinatal factors with neurologic outcome in the newborn and child. Current projects employing this material involve the investigation of seizure disorders and motor disability in twins, and the fetal heart rate monitoring by intermittent auscultation as related to neonatal and later neurologic outcome. A project on growth in cerebral palsy (CP), before and after birth, is planned.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02891-02 NEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Multiple Births and Cerebral Palsy in Western Australia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

Beverly Petterson, M.D., Fiona Stanley, M.D., Linda Watrson, Western Australian Research Institute for Child Health, Princess Margaret Hospital for Children, Perth 6001, Western Australia

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
  [x] (a1) Minors
  [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A population-based study was performed in Western Australia, identifying children with cerebral palsy born in the 1980s and linking information on plurality ascertained through vital statistics of the Health Department of Western Australia. This work confirmed earlier findings from the NEB and extended them to include triplets. The chance that a triplet pregnancy will produce a child with cerebral palsy is 47 times greater than for a singleton pregnancy.

An International Collaborative Study of Childhood Neurologic Disorders in Multiple Births has begun. A colleague from Western Australia will come to the NEB for six months to work on the study.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02892-02 NEB

PERIOD COVERED
October 1, 1993 through September 30 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The EEG as a Predictor in Febrile Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS
	Sherrie Emoto, Ph.D.	Staff Fellow	BFSF, DIR, NINDS
Others:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS
	Deborah Hirtz, M.D.	Medical Officer	DNB, NINDS

COOPERATING UNITS *(if any)*
Nikola Sofijanov, M.D. , Milutin Dukovski, M.D., Marija Kuturek, M.D., Pediatric Clinic of the University of Skopje, Macedonia (Former Yugoslavia)

LAB/BRANCH
Neuroepidemiology Branch and Biometry and Field Studies Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
- [x] (a) Human subjects
  - [x] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

From a population study in Macedonia, all children with febrile seizures were evaluated in one child neurology unit, histories, physical examinations, and electroencephalograms (EEGs) recorded, and children followed for two years

We are examining the utility of the electroencephalogram as a predictor of recurrence of febrile seizures in a defined population. In earlier work we explored the relationship of chinical characteristics at study entry with results of first study EEG. In work now underway we are examining those characteristics and the initial EEG as predictors of outcome.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02073-21 NB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nuclear Magnetic Resonance (Imaging and Spectroscopy)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Giovanni Di Chiro, M.D.	Chief	NB, CNP, DIR, NINDS
Others:	R.A. Brooks, Ph.D.	Staff Physicist	NB, NINDS
	J.R. Alger, Ph.D.	Research Biochemist	NB, NINDS
	C Pierpaoli, M.D.	Visiting Fellow	NB, NINDS
	G. Tedeschi, M.D.	Exchange Scientist	NB, NINDS
	A. Barnett, Ph.D.	Research Physicist	NB, NINDS
	J. Vymazal, M.D., Ph.D.	Visiting Fellow	BEIP, NCRR*

COOPERATING UNITS *(if any)*

In vivo NMR Research Center; Diagnostic Radiology Department; BEIP, NCRR; DMNB, NINDS; SB, NINDS; Albert Einstein College of Medicine, NY

LAB/BRANCH

Neuroimaging Branch

SECTIONS

Clinical Studies and MR Spectroscopy

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 7.8	PROFESSIONAL: 7.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our NMR imaging research is developing along the following lines: (a) Proton-MR spectroscopic imaging (^{1}H-MRSI) in normal controls as well as in patients with tumors, stroke, lipid storage diseases, Alzheimer's disease and other degenerative conditions; (b) diffusion-perfusion imaging and (^{1}H-MRI) in patients with stroke; (c) comparing clinical MRI imaging results with those of PET; (d) analysis of the signal intensity from critical areas (basal ganglia, substantia nigra) in patients affected by a variety of movement disorders; (e) diffusion-perfusion imaging plus proton MR spectroscopy in experimental cerebral ischemia in cats and rats; and (f) *in vitro* studies of ferritin's NMR properties.

---

*Continued:

R. Raman, M.D.	Senior Staff Fellow	NB, NINDS
R. Dickerson, M.D.	Clinical Associate	NB, NINDS
C. Baumgarner, Ph.D.	Chemist	NB, NINDS
A. Bertolino, M.D.	Special Volunteer	NB, NINDS
A. Righini, M.D.	Visiting Fellow	NB, NINDS
R. Miletich, M.D., Ph.D.	Sr. Clinical Investigator	NB, NINDS
R. O. Brady, M.D.	Chief	DMNB, NINDS
N. Barton, M.D,	Section Chief	DMNB,NINDS
J.M. Hallenbeck, M.D.	Chief	SB, NINDS
T.J. De Graba, M.D.	Medical Researcher	SB, NINDS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02315-17 NB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Positron Emission Tomography

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

P.I.	Giovanni Di Chiro, M.D.	Chief	NB, CNP, DIR, NINDS
Others:	R.A. Brooks, Ph.D.	Staff Physicist	NB, NINDS
	R. S. Miletich, M.D., Ph.D.	Sr. Clinical Invest.	NB, NINDS
	R. Raman, M.D.	Sr. Staff Fellow	NB, NINDS
	M. Quarantelli M.D.	Special Volunteer	NB, NINDS
	I. Bicik, M.D.	Special Volunteer	NB, NINDS
	P. Jacob, M.D.	Clinical Associate	NB, NINDS*

COOPERATING UNITS (*if any*)

CNB, NINDS; DIR, NINDS; NM, CC; BEIP, NCRR; SNB, NINDS; D,NIDDK.

LAB/BRANCH

Neuroimaging Branch, CNP, DIR

SECTIONS

Clinical Studies and Experimental PET

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.1	PROFESSIONAL: 2.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[x] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Positron emission tomography (PET) is a nuclear medicine technique which allows us to obtain some anatomic data (e.g., axial, coronal or sagittal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate). The unique property of PET is that it provides physiologic and pathophysiologic information not available with any other imaging procedure. Using a variety of radiopharmaceuticals as tracers, we have investigated with PET, brain tumors and movement disorders (Parkinson's disease, in particular). New information has been gathered, both in the basic and in the clinical (patient management) areas.

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* Continued:

D. Laske, M.D.	Medical Staff Fellow	SN, NINDS
C. Ram, M.D.	Visiting Scientist	SN, NINDS
E.H. Oldfield, M.D.	Chief	SN, NINDS
C.V. Kufta, M.D.	Staff Physician	SN, NINDS
I. Simpson, M.D.	Associate Chief	D, NIDDK
D. Accili, M.D.	Visiting Scientist	D, NIDDK
M. Hallett, M.D.	Clinical Director	CNP, NINDS
I.J. Kopin, M.D.	Director	DIR, NINDS

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02202-19 NIB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Immunogenetic Studies in Patients with Multiple Sclerosis and Other CNS Diseases*

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
Others:	Mary E. Smith, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS
	Tanya Lehky, M.D.	Senior Assistant Surgeon	NIB	DIR	NINDS
	Michael Racke, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS
	Lael Stone, M.D.	Clinical Associate	NIB	DIR	NINDS
	Steven Jacobson, Ph.D.	Section Chief	NIB	DIR	NINDS
	Peter Calabresi, M.D.	Clinical Associate	NIB	DIR	NINDS

COOPERATING UNITS (*if any*)

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.3	PROFESSIONAL: 1.8	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [X] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The overall goal of this project is to assess genetic and immunological factors that contribute to the pathogenesis of neurological disease. Particular attention is focused on multiple sclerosis (MS), since this disease is thought to have an immunological basis. Both genetic and immunological factors are being examined in patients with well-characterized clinically definite MS. In addition to studies in sporadic patients, members of multiplex families with multiple affected members and patients with identical or nonidentical twins either affected or nonaffected with MS are also being studied.

Various forms of immunosuppressive therapy are being examined in the treatment of MS. These studies employ MRI as a means for monitoring efficacy of treatment.

The clinical course and potential treatment of patients with HTLV-I associated myelopathy/tropical spastic paraparesis is being evaluated, since this disease may represent an example of virus-induced immunopathological disease. The presence of clinical disease is being correlated with the evidence of immunological abnormalities which may reflect an immunopathological process. New innovative therapies designed to reduce the number of activated T cells are now being initiated.

*(Formerly, "Immunological Studies in Patients with Multiple Sclerosis and Other CNS Diseases")

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02204-19 NIB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Mechanisms in Experimental Autoimmune Diseases of the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
Others:	Michael K. Racke, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS
	Mary E. Smith, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS

COOPERATING UNITS *(if any)*

Cedric S. Raine, Ph.D., Prof., Albert Einstein U.: Michael J. Lenardo, M.D., Chief, Development of the Immune System Unit, NIAID; Richard M. McCarron, Ph.D., Special Expert, SB, DIR, NINDS

LAB/BRANCH

Neuroimmunolgy, CNP

SECTION

Neurological Disease Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.5	PROFESSIONAL: 2.1	OTHER: 1.4

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Current work is focused on the chronic- relapsing model experimental allergic encephalomyelitis (EAE). This disease is produced by transfer lymphocytes sensitized to myelin basic protein (MBP) or proteolipid protein to syngenic mice. Neurologic dysfunction is characterized pathologically by inflammation and primary demyelination. The immunological mechanisms responsible for the initial episode and for the chronic disease are being investigated. Particular attention has focused on the role of activated T cells and regulatory mechanims occurring at the level of EAE lesion. Previous studies have demonstrated that transforming growth factor beta (TGF-β 1) can reduce the clinical severity of EAE even when given after the initial onset of disease. Recent studies have demonstrated that the administration of soluble MBP, given the following cell transfer or even after the onset of clinical disease, can also ameliorate clinical illness. It is thought that the mechanism for this effect is the induction of apoptosis or programed cell death. T cells which are activated and encountered antigen go without a second signal i.e., IL-2 may be induced to undergo apoptosis. Finally, the role of superantigens such as staphylococcal enterotoxin B have been shown to produce sufficient activation of T cells previously sensitized to MBP to allow transfer of disease. These findings indicate nonspecific activation factors could contribute to autoimmune disease process.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02205-19 NIB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between the Human Immune System and Antigens in the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
Others:	William E. Biddison, Ph.D.	Section Chief	NIB	DIR	NINDS
	Peter Calabresi, M.D.	Clinical Associate	NIB	DIR	NINDS
	Clara Pelfrey	Special Volunteer	NIB	DIR	NINDS
	Roland Martin, M.D.	Special Volunteer	NIB	DIR	NINDS
	Marco Vergelli, M.D.	Visiting Associate	NIB	DIR	NINDS
	Amy Lovett, Ph.D.	IRTA Fellow	NIB	DIR	NINDS

COOPERATING UNITS *(if any)*

John R. Richert, M.D., Assoc. Prof., Georgetown U.; Diane Griffin, M.D., Ph.D., Prof., Dept. Neurology, Johns Hopkins U.; Rhonda R. Voskuhl, M.D., Clinical Associate, Molecular Genetics Section, NINDS

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NINDS, NIH, Besthesda, Maryland 20892

TOTAL STAFF YEARS: 5.53	PROFESSIONAL: 3.33	OTHER: 2.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects   ☒ (b) Human tissues   ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to examine the manner in which immunologic mechanisms may contribute to diseases of the nervous system. The cellular and humoral immune response to putative antigens and possible immunopathologic disease such as multiple sclerosis (MS) are being studied. Included in these studies have been examinations of the immune response to viruses which can commonly infect the nervous system and which could be related to the induction of immunopathologic disease processes. In addition, the immune response to antigens of myelin such as myelin basic protein (MBP) and proteolipid protein (PLP) which may represent targets of immune-mediated diseases of myelin, has been studied. T-cell response to these antigens is being examined in patients with clinically definite MS and healthy controls, in members of MS multiplex families, and in identical and nonidentical twins, either concordant or discordant for MS. Various parameters of the T-cell response to myelin antigens is being examined, including frequency, peptide specificity, and HLA restriction.

The T-cell response to myelin antigens other than the 18.5kDa form of MBP have been examined. These include the protein encoded by the exon 2, which is expressed in the 21.kDa form of MBP found in developing myelin, the C8 form of MBP that has 6 citrulline substitutions for alanine in the 18.5kDa form, and PLP. A substantial T-cell response to exon 2 and to C8 has been demonstrated indicating that, although these proteins are found in extremely low concentrations of the nervous system, they appear to be immunogenic and could potentially represent target autoantigens in autoimmune diseases of the nervous system. Studies of PLP have employed synthetic peptides and have demonstrated a response that appears to be immunodominant to portions of the PLP molecule. No substantial differences in these responses between patients and healthy controls have been noted. In addition, the T-cell response to viruses which have been implicated previously in MS such as measles virus are being examined. The parameters of the normal T-cell response following natural infection or vaccination are being studied. The humoral and cellular immune response to measles virus is being examined in individuals previously vaccinated, but lacking evidence of immunity.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02603-11 NIB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Lymphoid Cell-Cell Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Biddison, Ph.D.	Section Chief	NIB	DIR	NINDS
Others:	Ursula Utz, Ph.D.	Visiting Fellow	NIB	DIR	NINDS
	Tomiko Tsuchida, M.D., Ph.D.	Visiting Fellow	NIB	DIR	NINDS
	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS

COOPERATING UNITS *(if any)*

John E. Coligan, Ph.D., Chief, Biological Resources Branch, DIR, NIAID

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.83	4.28	2.55

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[X] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general objective of this project is to define the mechanisms by which human lymphoid cells interact with antigen-presenting cells in order to produce and regulate immune responses. Over the past year, there have been two major efforts underway that are targeted on this objective: 1) dissection of the molecular basis of viral peptide binding and presentation for T-cell recognition by HLA class I molecules; and 2) analysis of expressed T-cell repertoires specific for myelin basic protein (MBP) in MS patients. The principle findings are as follows: 1) isolation and sequencing of endogenous peptides bound to the HLA class I molecules HLA-A1, A3, and HLA-B8 has permitted identification of specific combinations of peptide anchor residues which can be used to successfully predict immunogenic T-cell epitopes within viral protein sequences that are presented by each of these HLA class I molecules; and 2) analysis of T-cell receptor usage in T-cell responses to MBP by genetically identical twins who are concordant or discordant for MS indicates that disease severity may be associated with increased heterogeneity of MBP-specific T cells and could reflect an impaired ability of the immune system to down-regulate these anti-self responses.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02817-05 NIB

PERIOD COVERED

October 1, 1993 through Septmber 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Involvement of Human Retrovirus Associated with Chronic Neurologic Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steven Jacobson, Ph.D.	Section Chief	NIB	DIR	NINDS
Others:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
	Tanya Lehky, M.D.	Senior Assistant Surgeon	NIB	DIR	NINDS
	Michael Levin, M.D.	Special Volunteer	NIB	DIR	NINDS
	Taketo Kawanishi, M.D.	Visiting Fellow	NIB	DIR	NINDS

COOPERATING UNITS (if any)

William Blattner, M.D., Chief, VES, NCI; Gene Shearer, M.D., Section Chief, EIB,Thomas Waldmann M.D., Chief, MET Branch, NCI; Anthony Fauci, M.D., Director, NIAID

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.18	PROFESSIONAL: 3.28	OTHER: 1.90

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [X] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The human T lymphotropic virus type I (HTLV-I) is associated with a chronic-progressive myelopathy known as HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Since this disorder is clinically similar to the chronic-progressive form of multiple sclerosis (MS), understanding the pathogenesis of a neurologic disease with a known viral etiology such as HAM/TSP will help define similar mechanisms in the pathogenesis in MS, a disease of unknown etiology. Four major areas of research are being targeted that address the pathogenesis of HTLV-I in HAM/TSP: (1) host immune response in the HAM/TSP disease process; in particular, the role of CD8+, HTLV-I specific and HLA class I-restricted cytotoxic T lymphocytes (CTL); (2) detection of human retroviral sequences in the central nervous system and lymphoid organs of HAM/TSP patients both *in situ* and *in vivo*; (3) demonstration of HTLV-I- specific T-cell responses to immunodominant synthetic peptides of HTLV-I from HTLV-I seronegative individuals at risk for exposure to HTLV-I, and; (4) molecular characterization of human retroviruses isolated from patients with HAM/TSP and other chronic-progressive neurologic diseases. The major findings of these studies are: (1) demonstration CD8+ CTL directly isolated from PBL or CSF of HAM/TSP patients that are specific for immunodominant peptides of the tax region of HTLV-I and are restricted to particular HLA alleles; (2) exceptionally high precursor frequencies were demonstrated to these peptides in the range of 1 in 75 to 1 in 300 C8+ cells; (3) HTLV-I tax mRNA signals were detected in spinal cord lesions of HAM/TSP patients; (4)*in situ*-PCR was developed and successfully amplified HTLV-I tax DNA from PBL to HAM/TSP patients; (5) HTLV-I-T-cell responses to synthetic peptides of HTLV-I could be demonstrated from HTLV-I seronegative, PCR negative individuals known to be exposed to this virus; (6) HTLV-I molecular sequences were identified in an HTLV-I seronegative individual with a chronic-progressive neurologic disease; and (7) HTLV-II has been unequivocally identified, both molecularly and immunologically, in an individual with a chronic myelopathy indistinguishable from HAM/TSP. These results continue to define the role of human retroviruses that are associated with chronic-progressive neurologic disease and the host immune responses to these agents that may be involved in the pathogenesis of these disorders.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02831-04 NIB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Regulation of Class I Major Histocompatibility Complex Gene Expression in the Central Nervous System*

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul D. Drew, Ph.D.	Senior Staff Fellow	NIB	DIR	NINDS
Others:	Kevin G. Becker, Ph.D.	Senior Staff Fellow	NIB	DIR	NINDS
	William E. Biddison, Ph.D.	Section Chief	NIB	DIR	NINDS

COOPERATING UNITS (*if any*)

Keiko Ozato, Ph.D., Laboratory of Molecular Growth Regulation, NICHD

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.05	PROFESSIONAL: 2.05	OTHER: 1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to understand the molecular mechanisms which regulate MHC class I gene expression in cells of the central nervous system. Over the past year, there have been three major efforts underway that are targeted on this objective. These are the identification of the mechanism of transcription factor control of beta 2-microglobulin and MHC class I gene expression in (1) mouse brain; (2) mouse embryonal carcinoma cells treated with retinoic acid; and (3) human fetal glial cells infected with HIV-1. The principle findings are: (1) the absence of MHC class I and beta2-microglobulin gene expression in the brain is due, at least in part, to the absence of transcription factor binding to prometer elements of these genes; (2) retinoic acid treatment of embryonal carcinoma cells induces heterodimers of NFκB p50/p65 to bind to MHC class I enhancers, resulting in increased MHC class I gene expression; and (3) reactivation of HIV-1 gene expression induced by TNF-alpha and IL-1 beta in human fetal glial cells involves the binding of NFκB p50/p65 to the HIV-1 LTR.

*(Formerly, "Regulation of Class II Major Histocompatibility Complex Genes in the CNS")

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02853-03 NIB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Examination of Natural History and Therapy of Multiple Sclerosis Using MRI

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
Others:	Michael K. Racke, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS
	Tanya J. Lehky, M.D.	Senior Assistant Surgeon	NIB	DIR	NINDS
	Lael Stone, M.D.	Clinical Associate	NIB	DIR	NINDS
	Peter Calabresi, M.D.	Clinical Associate	NIB	DIR	NINDS
	Mary E. Smith, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS

COOPERATING UNITS (*if any*)

Joseph A. Frank, M.D., Director, LDDR, OD, Paul Albert, Ph.D., BFS, DIR, NINDS; Charles DeCarli, M.D., Epilepsy Research Branch, NINDS.

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.25	PROFESSIONAL: 2.1	OTHER: 2.15

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to use magnetic resonance imaging (MRI) to examine the natural history and potential therapeutic approaches in multiple sclerosis (MS). Emphasis has been placed on investigation of the early MS lesion which is characterized by enhancement on T1 weighted MRI images following administration of gadolinium-DTPA (GD). Results from initial studies have indicated that MS can be an active disease, even during periods of remission in the early, relapsing-remitting phase of the illness. The correlation between the frequency or area of Gd-enhancing lesions occurring in the cerebrum are clinically silent. Episodes of worsening tend to occur during periods of increased disease activity as evidenced by increased frequency or area of enhancing lesions. The clinical symptoms and signs generally are due to lesions occurring in the spinal cord or brain stem concurrently with the increased activity in the cerebrum. These results demonstrate a correlation between clinical worsening and periods of increased disease activity occurring in the cerebrum and indicate that the regulation of disease activity as measured by Gd enhancement seems similar in the cerebrum and spinal cord. Examiniation of the pathological changes occurring in conjunction with Gd enhancement indicate an acute inflammatory process with prominent perivascular cuffs of lymphocytes. These findings support the hypothesis that Gd enhancement represent the initial step in lesion development.

The ability to use MRI as an outcome measure in clinical trials has been examined in a baseline, versus treatment trial design. The results of this study indicate response heterogeneity in patients treated with cyclosporine. Three patients out of ten had a dramatic reduction in MRI parameters, which based on analysis of the natural history data, is significant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02886-02 SB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Quantification of Neurologic Deficit Progression in Acute Stroke Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I:	T. DeGraba, M.D.	Senior Staff Fellow	SB, NINDS
Others:	J. Hallenbeck, M.D.	Chief	SB, NINDS

COOPERATING UNITS *(if any)*

B. Kelly, M.D. and A. Dutka, M.D., Dept. of Neurology, National Naval Medical Center, Bethesda, MD

LAB/BRANCH

Stroke Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0.76	0.43	0.33

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects   [ ] (b) Human tissues   [ ] (c) Neither

[ ] (a1) Minors

[X] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Stroke has been traditionally regarded as a catastrophic event in which maximal damage to brain tissue occurs almost immediately. Recently, clinical and animal research has revealed that the ultimate degree of tissue damage in a stroke is not determined in the first few minutes but instead evolves over a period of hours to days. Amplification of excitotoxic neurotransmitter release, progressive intracellular calcium accumulation, blood-brain barrier compromise, and regional inflammation all may play a role in delayed neuronal death. In conjunction with monitoring physiologic variables, including blood pressure and oxygen saturation, careful observation of clinical neurologic progression may provide an understanding of the "window of opportunity" for acute interventional therapy. In meeting the primary objective of this study 75 consecutive stroke patients were admitted to the National Naval Medical Center (NNMC) within 24 hr of the onset of cerebral ischemic symptoms . A record was kept of the progression of clinical deficits over the first 48 hr. A standardized examination (the NIH Stroke Scale) was performed on admission and every 8 hr for 48 hr. The patients were monitored in the neurology ICU with a continuous recording of blood pressure, heart rate, and oxygen saturation obtained over this same period. The goal of analysis was to identify the percent of NNMC patients who would develop significant progression in the acute postischemic period. Subgroup analysis showed that patients with initial NIH stroke scores of $\geq$ 10 were more likely to show progression ($P = 0.001$) than those with scores of < 10. Additionally, the presence of atrial fibrillation and a higher minimal arterial blood pressure were also associated with a higher risk of progression ($P = 0.007$ & $P = 0.042$ respectively.)

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02887-02 SB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Activation of Cytokines, Leukocytes, and Endothelium After Acute Cerebral Ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I:	T. DeGraba, M.D.	Senior Staff Fellow	SB, NINDS
Others:	J. Hallenbeck, M.D.	Chief	SB, NINDS
	R. McCarron, Ph.D.	Research Microbiologist	SB, NINDS
	M. Spatz, M.D.	Section Chief	SB, NINDS
	L. Penix, M.D.	Fellow	SB, NINDS

COOPERATING UNITS *(if any)*
B. Kelly, M.D., Dept. of Neurology NNMC, V. Aletich, M.D., Dept. of Radiology, M. Foust, M.D., N. Bakalar, M.D., T. Porter, M.D., Dept. of Psychiatry, NNMC

LAB/BRANCH
Stroke Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.95	PROFESSIONAL: 0.62	OTHER: 0.33

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[X] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Effective management of acute stroke patients has remained elusive to the present date. Recent evidence indicates that increased cytokine levels and leukocyte and endothelial cell activation may play a major role in secondary neuronal injury after acute focal cerebral ischemia. The purpose of this investigation is to more clearly characterize the role of the inflammatory response after ischemic injury in humans with regard to its causal influence on secondary neuronal injury and predictive value for long-term functional outcome. Blood samples are drawn from acute stroke patients on admission and serially for the first 7 days. Serial neurologic exams are being performed and MRI scan of the head is done within the first 3 days of admission to determine infarct size. Depression scales are done within the first 14 days. All patients are being seen at 90 days after the ischemic event for follow up at which time blood cytokine levels, neurologic outcome scales, and depression scales are being performed. Through analysis of the advent and duration of cytokine activation, we hope to establish a correlative relationship between the postischemic inflammatory response and neuronal injury. Given the published work demonstrating neuronal protection after ischemia in animal models with antagonists of leukocyte activation and of the inflammatory pathways, we expect these results to establish a temporal window for future drug trials in reducing infarct size after acute stroke. In addition, since clinical outcome in stroke is also dependent on rehabilitation effort, the incidence of depression in stroke patients becomes an important variable in long-term outcome. Thus, we will observe the incidence of depression in stroke patients as it relates to the volume and location of cerebral infarction. A novel approach of correlating sleep architecture in stroke patients with the incidence of mood disturbance will be performed by obtaining a polysomnogram in patients 3-6 months after the ischemic event. A comparison will be made between patients with and without depression. Polysomnograms will also be compared against those of patients with primary depression (who display a very characteristic sleep pattern). It is hypothesized that the mood disturbance in stroke patients may actually be a result of altered sleep patterns caused by the neuronal injury. This may lead to a new understanding of the etiology of mood disorders in stroke patients and aid in their treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02888-02 SB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cytokine, Leukocyte and Endothelium Activation in Risk Factors for Stroke

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I:	T. DeGraba, M.D.	Senior Staff	SB, NINDS
Others:	J. Hallenbeck, M.D.	Chief	SB, NINDS
	R. McCarron, Ph.D.	Research Microbiologist	SB, NINDS
	M. Spatz, M.D.	Section Chief	SB, NINDS
	L. Penix, M.D.	Fellow	SB, NINDS

COOPERATING UNITS *(if any)*
B. Kelly, M.D., A. Dutka, M.D., Dept. of Neurology, NNMC, V. Aletich, M.D., Dept of Radiology NNMC; C. Cunningham, M.D., Dept. of Vascular Surgery, R. Hargraves, M.D., Dept. of Neurosurgery, NNMC

LAB/BRANCH
Stroke Branch

SECTION
Clinical Investigation Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 1.05	PROFESSIONAL: 0.71	OTHER: 0.34

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[x] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major risk factors which are associated with an increased incidence of stroke have been known for many years. However, the basic mechanisms by which these factors lead to the increased risk are not fully understood. Preliminary studies indicate that activation of the immune system by risk factors for stroke (hypertension, hypercholesterolemia, diabetes and age) increases the risk of endothelial activation and the formation of intravascular thrombosis. By measuring the levels of cytokine and monocyte, macrophage and endothelial cell activation in the stroke-prone population and age matched controls without risk factors, an attempt will be made to characterize those factors which potentially increase the risk for activation of brain vessel endothelium as well as preparing the brain tissue (including the cerebral vasculature) for a hyperactive inflammatory response to an ischemic insult. In addition, although disease is a major cause of stroke in the U.S., no radiographic findings related to the stenosis nor specific morphologic features of the atherosclerotic plaque have been useful in predicting which will become symptomatic and which will remain asymptomatic. In this study, we are analyzing carotid endarterectomy surgical specimens from symptomatic and asymptomatic patients for leukocyte adhesion molecules on the plaque endothelial cells using immunofluorescence staining. Blood drawn at the time of pre-operative testing is being examined for leukocyte and endothelial cell activation by fluorescence activated cell sorting (FACS) and baseline cytokine levels. It is hypothesized that the local release of cytokines and the expression of endothelial cell surface leukocyte receptors play a major role in the conversion of an asymptomatic plaque to a symptomatic one. Understanding the role of cytokines, leukocyte activation, and endothelial interaction in promoting the cerebral ischemic state may lead to a novel approach in future stroke prevention regimens.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02885-02 SB

PERIOD COVERED

October 1, 1993, 1992 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Regulation of Gene Activity in Astrocytes

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

P.I.	Michael Brenner, Ph.D.	Special Expert	SB, NINDS
Others:	Qi Zhang, Ph.D.	Visiting Fellow	SB, NINDS
	John Hallenbeck, M.D.	Chief	SB, NINDS

COOPERATING UNITS (*if any*)

A.H. Koeppen, MD, (VAMC, Albany, N.Y.) X.Liu, MD, D-L Yao, MD, (LENP, NINDS, NIH); A Messing VMD, PhD(Sch Vet Med, Univ Wis, Madison, WI); J Schwartz, PhD (CNB, NINDS); S-J Kim, PhD (CPCP, NCI) AJ Tobin, PhD.(UCLA), B. Pessac , M.D. (CNRS, Paris France)

LAB/BRANCH

Stroke Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.7	PROFESSIONAL: 1.5	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Current studies of the central nervous system (CNS) are assigning an increasing number of activities to astrocytes, many of which are potentially relevant to stroke. However, nearly all of these suggested functions are based on observed correlations, and many of these were made on cultured cells, whose properties may differ from those *in vivo*. As an alternative approach to understanding astrocyte function, we are studying transcriptional regulation of the human gene encoding glial fibrillary acidic protein (GFAP), the major component of astrocyte intermediate filaments. By studying GFAP transcription, insights may be gained into mechanisms governing development, reaction to injury, and cell specificity. A second goal is to use identified astrocyte-specific transcription elements to direct expression of other genes in astrocytes. This enables testing of the roles of specific factors in CNS function, and may produce disease models. Transcriptional studies have focused primarily on identifying factors that act at a consensus AP-1 site that is essential for GFAP transcription. Since proteins encoded by the *jun* and *fos* proto-oncogene families are known to modulate transcription via AP-1 sites, their presence in a GFAP expressing astrocytic cell line was examined. Analyses included gel mobility shift assays, "shift Westerns" and detection of the specific mRNAs by Northern analysis. Preliminary results show a correlation between GFAP transcription and the presence of c-Jun, JunD, and Fra-2, but not with JunB, c-Fos, FosB or Fra-1.

Several results have been obtained with mice carrying transgenes driven by GFAP regulatory regions. One study has provided additional support for our previous suggestion that astrocytes in different regions of the CNS use different regulatory elements of the GFAP gene to control its expression. Another has shown that overexpression of TGF-β1 in astrocytes produces a severe, communicating hydrocephalus whose penetrance is dependent on the genetic background of the host. A third study has demonstrated the importance of astrocytes in development of the CNS. Mice carrying the herpes simplex virus thymidine kinase (TK) gene were treated with gancyclovir, an anti-herpes drug that is innocuous unless converted to a toxic product by the TK. Treated mice were ataxic, and displayed complete cerebellar disruption, accompanied by a reduction in the amount and organization of myelin.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02856-03 SB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hibernation - A New Approach to Stroke Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J. M. Hallenbeck, M.D.	Chief	SB, NINDS
Others:	K. Tasaki, M.D.	Guest Researcher	SB, NINDS
	M. Brenner, Ph.D.	Special Expert	SB, NINDS
	R. Meija, M.D.	Guest Researcher	SB, NINDS
	R. McCarron, Ph.D.	Research Microbiologist	SB, NINDS
	M. Spatz, M.D.	Research Medical Officer	SB, NINDS

COOPERATING UNITS *(if any)*
L. Sokoloff, M.D., C. Kennedy, M.D. G. Dienel, Ph.D., C. Smith, Ph.D.LCM, NIMH; H. Gainer, Ph.D., H. Jaffe, Ph.D., LNC/NINDS

LAB/BRANCH
Stroke Branch

SECTION
Clinical Investigation Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.19	PROFESSIONAL: 2.64	OTHER: 1.55

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts to develop effective measures for the treatment of stroke have generally been based on the implicit assumption that one, or at the most several, factors control the progressive brain injury that occurs during the early hours of focal brain ischemia. Postischemic progression of brain damage appears to be extremely multifactorial. There is a finite probability that the assumption underlying most therapeutic stroke trials that seek to identify a dominant or controlling factor that determines post-ischemic progression of brain damage is incompatible with the fundamental nature of the problem. Postischemic progression of brain damage may be the result of a constellation of minor causes and the quest for a dominant or controlling cause would then be ultimately futile. Unconventional approaches may be required to arrest cellular destruction in brain ischemia.

This project continues to investigate mammalian hibernation, a state of natural tolerance to severely reduced blood flow and oxygen delivery. Efforts to isolate and identify the factor or factors that regulate the controlled metabolic depression and tolerance of profound brain ischemia that forms the essence of natural hibernation are in progress. Such factors with pleiotropic effects may have benefit in the treatment of progressive brain damage in human stroke that is characterized by loss of homeostatic control due to activation of a multitude of pathophysiological postischemic events. The existence of regulatory factors in hibernation is supported by several findings that render passive submission to the effect of ambient temperature unlikely: (1) The onset and rate of development of bradycardia and reduced oxygen consumption during the transition to hibernation is rapid and precedes a more gradual drop in body temperature. (2) Regulation of enzyme function and gene expression that contributes to preservation of homeostasis during hibernation has been demonstrated. (3) Artificially induced hypothermia leads to rapid death in animals otherwise able to tolerate the same degree of hypothermia during natural hibernation. The identification of these putative control mechanisms may enable us to prevent or minimize the breakdown of homeostasis and cellular damage in cerebral ischemia in other species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02324-18 SB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Blood-Brain Barrier: *In Vitro* Model for the Study of Cerebrovascular Endothelial Permeability

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R.M. McCarron, Ph.D.	Microbiologist	SB, NINDS
Others:	M. Spatz, M.D.	Section Chief	SB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Stroke Branch

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was temporarily suspended for the fiscal year 1994.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02865-03 SB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between Cerebrovascular Endothelial Cells and Blood Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R.M. McCarron, Ph.D.	Microbiologist	SB, NINDS
Others:	J.M. Hallenbeck, M.D.	Chief	SB,NINDS
	M. Spatz, M.D.	Section Head	SB,NINDS
	Y. Yasuma, M.D.	Guest Researcher	SB,NINDS

COOPERATING UNITS *(if any)*

A-L. Siren, Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 2.35	PROFESSIONAL: 2.05	OTHER: 0.30

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects [ ] (b) Human tissues [X] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These experiments investigate interactions between cerebromicrovascular endothelial cells (EC) which constitute the blood-brain barrier (BBB) and peripheral blood cells and/or components. Adhesive interactions between circulating monocytes and EC were examined *in situ* and *in vitro*. These experiments utilized (10-16 wk) spontaneously hypertensive rats (SHR), stroke-prone SHR (SHR-SP), normotensive Wistar-Kyoto (WKY), Sprague-Dawley (SD) and Fisher 344 (F344) rats, and old (2-3 yrs) SD and F344 rats. The *in vivo* experiments assessed the number of perivascular macrophages (ED2-positive cells) in blood vessels of perfusion-fixed frozen brain sections. The data demonstrated increased numbers of perivascular macrophages in cerebral intraparenchymal vasculature of hypertensive and aged rats. Experiments also demonstrate that LPS-induced (i.v. and i.c.v.) release of von Willebrand factor (vWF) in SHR rats was significantly greater ($p<0.05$) than in WKY rats. Stroke risk factors such as hypertension may predispose endothelium to be more responsive to agonist-stimulated secretion of vWF (which is important in homeostasis and thrombosis). *In vitro* experiments using cultured cerebromicrovascular EC derived from SHR and WKY rats demonstrated increased monocyte adhesivity to cytokine- or LPS-treated EC. The level of up-regulation of monocyte adhesion to SHR EC was significantly greater than to similarly treated WKY EC. The results suggest that stroke risk factors hypertension and advanced age are associated with: (1) an increased adhesion of monocytes to endothelium (*in vivo*) and EC monolayers (*in vitro*); (2) a hyper-responsive state of monocytes; and (3) an increased tendency of endothelium to convert to a procoagulant surface. All the above findings implicate factors such as cytokines and LPS in disorders involving recruitment, attachment and/or transvascular migration of blood cells at sites of inflammatory responses. The data indicate how advanced age and hypertension may act as risk factors for stroke (i.e., increase the likelihood of interactions between monocytes and endothelium leading to local thrombosis or hemorrhage).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02776-06 SB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Production of Experimental Allergic Encephalomyelitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R.M. McCarron, Ph.D.	Microbiologist	SB, NINDS
Others:	M. Spatz, M.D.	Section Chief	SB,NINDS

COOPERATING UNITS (if any)

Dr. M.K. Racke, NIB, NINDS
Dr. R.S. Fujanami, Dept. Neurol., Univ. of Utah, Salt-Lake City, UT

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments were conducted to characterize cerebrovascular endothelial cells (EC) lines derived from SJL/J mice with respect to their infectibility with Theiler's murine encephalomyelitis virus and vaccinia virus. In addition, the capacity of these cells to function as antigen-presenting cells in immune responses to these murine viruses was also studied. The EC cell line had interferon-γ inducible expression of MHC antigen. Infection with Theiler's murine encephalomyelitis virus also influenced the expression of MHC molecules by these cells. The data indicated that these EC cultures could not stimulate T lymphocyte proliferation or function as targets for cytolysis by Theiler's murine encephalomyelitis cytolytic immune spleen cells. However, EC lines were able to present viral antigen to vaccinia virus immune spleen cells and act as targets for cytotoxic T cells from vaccinia virus immune mice. The data indicate cerebrovascular EC cultures are a valuable resource for the study of biology and immune response to murine viruses, such as Theiler's virus. These findings have important implications regarding pathogenic mechanisms of adoptively transferred experimental allergic encephalomyelitis (EAE) which has been shown to involve cerebrovascular EC.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02689-10 SB

PERIOD COVERED
October 1, 1993 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Endothelin and Prostanoid Production in Cerebromicrovascular Endothelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS
Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Stroke Branch

SECTION
Section of Neurocytobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was suspended for FY94 due to the very limited availability of human brain endothelial cells.

Publications:

Stanimirovic DB, Bacic F, Uematsu S, Spatz M. Profile of prostaglandins induced by endothelin-1 in human brain capillary endothelium. Neurochem Int 1993;23:385-93.

Spatz M, Stanimirovic D, Bacic F, Uematsu S, McCarron RM. Vasoconstrictive peptides induce endothelin-1 and prostanoids in human cerebromicrovascular endothelium. Am J Physiol 1994;266 (Cell Physiol 35): C654-60.

Spatz M, Stanimirovic D, McCarron RM. Vasoactive peptides and prostaglandin D2. J Auton Nerv Syst 1994; in press.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02357-16 SB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebral Ischemia: Neurotransmitters, Metabolism, and Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS

COOPERATING UNITS *(if any)*
B. Mrsulja, Inst. of Biochemistry, Fac. Med., Belgrade,Yugoslavia; N. Bertrand, Univ. Bourgoyne, Dijon, France

LAB/BRANCH
Stroke Branch

SECTION
Section of Neurocytobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of transient cerebral ischemia on cholinergic metabolism was investigated in different brain areas of adult and young gerbils subjected to bilateral carotid artery occlusion for 15 min alone or with various times of recirculation. Tissue acetylcholine (ACh) and choline (Ch) levels were assessed by HPLC and electrochemical detection. Ischemia led to a significant decrease in tissue ACh and increase in tissue Ch levels, affecting to a lesser extent the young gerbils. Blood recirculation induced a rapid early restoration of the tissue ACh levels in young animals whereas a tremendous rebound of ACh was observed in the adult group. After 2 hr of reflow, Ch levels were normalized in adult brain and significantly reduced in the young brain. These findings represent the first comparative study demonstrating an age-dependent difference between young and adult animals in the susceptibility of cholinergic neurotransmission to cerebral ischemia.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02623-11 SB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebral Ischemia and Edema: Biogenic Amines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Spatz, M.D.	Section Chief	SB	NINDS
Others:	R.M. McCarron, Ph.D.	Microbiologist	SB	NINDS
	Y. Yasuma, M.D.	Visiting Fellow	SB	NINDS
	N. Kawai, M.D.	Visiting Fellow	SB	NINDS

COOPERATING UNITS *(if any)*
B. Mrsulja, M.D., Inst. of Biochemistry, Fac. of Med., Belgrade, Yugoslavia; D. Stanimirovic, M.D., Ph.D., National Research Council of Canada, Ottawa, Canada

LAB/BRANCH
Stroke Branch

SECTION
Section of Neurocytobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.95	PROFESSIONAL: 0.55	OTHER: 0.40

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of nitro-L-arginine [(NLA), inhibitor of nitric oxide synthase (NOS) or L-arginine [(LA), precursor of nitric oxide (NO)] on cerebral blood flow (CBF), blood pressure (BP), and dopamine (DA) metabolism was investigated in the gerbil model of global ischemia. Inhibition of NOS by NLA in contrast to LA delayed the initial recovery of CBF and altered DA metabolism in brain ischemia/reperfusion. The data suggest that nitric oxide is involved in postischemic CBF recovery and NLA-induced CBF reduction in modulating DA metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02777-06 SB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Cerebromicrovascular Endothelium: Distinct Peptidergic Responses *in vitro*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS
Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS

COOPERATING UNITS *(if any)*

D. Stanimirovic, M.D., Ph.D., National Research Council of Canada, Ottawa, Canada

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.2	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The adhesion of circulating leukocytes on vascular endothelium is a prerequisite for their emigration to extravascular tissues. The data presented here demonstrate that intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) are constitutively expressed on endothelial cell lines derived from both human brain (HBEC) and umbilical cord veins (HUVEC). Lipopolysaccharide, tumor necrosis factor-α or interferon-γ treatment of both HBEC and HUVEC cell lines up-regulated the expression of these adhesion molecules in a time- and dose-dependent manner. These same pro-inflammatory factors also induced the expression of E-selectin on both HBEC and HUVEC cell cultures. Endothelins (ET-1, ET-2, and ET-3) also had a similar effect on the expression of all three adhesion molecules on HBEC. However, none of the endothelins had any effects on ICAM-1, VCAM-1, or E-selectin expression by HUVEC, despite the concomitant effects of the aforementioned factors on identical cultures. These results indicate that endothelial cells derived from various anatomic locations respond differently to the vasoactive peptide, endothelin, and implicate variations in the role(s) of endothelial cells derived from different anatomic locations in recruitment of blood cells at sites of inflammation.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02797-06 SB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cultures of Human Cerebromicrovascular Endothelium: Mechanisms of Endothelin Effects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	N. Kawai, M.D.	Visiting Fellow	SB NINDS
Others:	M. Spatz, M.D.	Section Chief	SB NINDS
	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH Bethesda,Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.35	0.95	0.40

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Capillary ATPase activity has been thought to control water-electrolytes homeostasis in the brain. Endothelin-1 (ET-1) has recently been implicated in changes of blood-brain barrier (BBB) permeability. This report describes receptor (ET_A)-mediated stimulation of ouabain-sensitive (OS) and ouabain-insensitive (OI) $^{86}Rb^+$ uptake (as a measure of Na^+-K^+-ATPase activity) by ET-1 and ET-3 in cultured rat brain capillary endothelial cells (BCEC). The uptake of $^{86}Rb^+$ (0.2 µCi/well) was determined in confluent BCEC (grown in 96-microwell plates) incubated in M199 with HEPES for 5 min at room temperature. A concentration-dependent increase of $^{86}Rb^+$ uptake induced by ET-1 or ET-3 (EC_{50} = 0.73 nM ± 0.17 and 12.89 ± 3.69, respectively] was inhibited by the ET_A receptor antagonist (BQ-123) but not by the ET_B receptor antagonist (IRL 1038). Ouabain (ATPase inhibitor) and bumetanide (Na^+-K^+-Cl^- cotransport inhibitor) decreased ET-1-stimulated $^{86}Rb^+$ uptake into BCEC by 35% and 65%, respectively. Complete inhibition was seen with both of these agents. Similar results were observed with PMA, a PKC agonist. Ameloride [5-(N-ethyl-N-isopropyl], inhibitor of the Na^+-H^+ antiporter, decreased both the OS and OI ATPase induced by ET-1, suggesting a linkage of the Na^+/H^+ exchanger with Na^+-K^+- ATPase and Na^+-K^+-Cl^- cotransport systems. The inhibition of ET-1-stimulated OS and OI $^{86}Rb^+$ uptake with staurosporin (PKC antagonist) indicates that ET-1-induced ATPase activity is mediated by PKC. These results suggest that the effect of ET-1 on capillary OS and OI Na^+-K^+-Cl^- systems may play a role in water-electrolyte disturbances in brain injury.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02795-06 SB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Cerebromicrovascular Endothelial Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS
Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS

COOPERATING UNITS *(if any)*

D. Stanimirovic, M.D., Ph.D., National Research Council of Canada, Ottawa, Canada

LAB/BRANCH

Stroke Branch

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.65	0.25	0.40

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human cerebromicrovascular endothelial cells (HBEC) in culture express high affinity ET_A receptors coupled to phospholipase C (PLC) activation. Pretreatment of HBEC with 1 µM dexamethasone for 24 hr decreased the number of ET-1 binding sites (B_{max}) on HBEC (96 fmol/mg protein vs. 57 fmol/mg protein) without changing the binding affinity (K_D) (101 pm vs. 92 pM) or displacing profile (ET-1 = ET-2>ET-3 >S6c). Dexamethasone-pretreated HBEC also exhibited a 40% reduction in the maximal ET-1 stimulated inositol triphosphate (IP_3) production, whereas the half-maximal stimulatory concentration (EC_{50}) was not affected. This effect of dexamethasone was concentration-dependent and most pronounced after 24 hr of pretreatment. The inhibitory effect of dexamethasone on the ET-1-induced IP_3 production was abolished by the glucocorticoid receptor antagonist cortexolone. In contrast, vasopressin-mediated IP_3 response in HBEC was not changed by dexamethasone. The cyclooxygenase inhibitors, indomethacin and acetylsalicylic acid, did not influence the ET-1-induced IP_3 production by HBEC. The down-regulation of ET_A receptors in HBEC by dexamethasone may represent one of the mechanisms involving the described effects of glucocorticoids on cerebromicrovascular function (i.e., changes in blood-brain barrier properties, secretion of vasoactive factors, vascular morphogenesis, etc.).

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02860-03 SB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Observations on Audiogenic Seizures in Rats Following Cardiac Arrest Cerebral Ischemia.

PRINCIPAL

P.I:	I. Klatzo, M.D.	Senior Scientist	SB/NINDS

COOPERATING UNITS *(if any)*
L.P. Penix, M.D., Epilepsy Research Branch, NINDS

LAB/BRANCH
Stroke Branch

SECTION
Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS 0.20	PROFESSIONAL: 0.10	OTHER: 0.10

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies on ther susceptibility to audiogenic seizures (AuSz), which regularly develop 24 hr after subjecting Sprague-Dawley rats to cardiac arrest cerebral ischemia (CACI), revealed a correlation in a chronological profile between onset of seizures, changes affecting the GABAergic terminals and loss of $GABA_A$ inhibition in the hippocampus as assayed by paired-pulse stimulation (PPS) testing. The cessation of susceptibility to AuSz approximately 1 month after ischemia appears to coincide with vigorous sprouting and new formation of GABAergic terminals and return of the PPS to a normal pattern. Studies on defining sites and mechanisms of GABAergic disinhibition are associated with evaluation of how much seizures may contribute to ischemic injury.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02832-04 SB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunochemical Observations on Neurotransmitter Changes in Global Cerebral Ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I:	I. Klatzo, M.D.	Senior Scientist	SB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Stroke Branch

SECTION
Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFFYEARS: 0.20	PROFESSIONAL: 0.10	OTHER: 0.10

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Immunohistochemical observations on GABA and glutamate decarboxylase (GAD) in rats subjected to cardiac arrest cerebral ischemia (CACI) revealed strikingly early changes in the immunoreactivity of GABAergic neuronal elements expressed in the widespread swelling and increased GABA and GAD immunostaining of GABAergic terminals and boutons. These changes appeared to be generally reversible with the exception of the nucleus reticularis thalami (NRT) which showed 80% neuronal loss. GABAergic terminals in the adjacent ventral thalamic nuclei (VTN) showed, approximately 7 days after their initial disintegration, a sprouting of new terminals, which reached its peak 1 month after ischemia. This coincided with the cessation of audiogenic seizures and the return to the normal paired-pulse stimulation patterns in the hippocampus, indicating a return of $GABA_A$ inhibitory function. The hybridization assays with GAP-43 revealed strong mRNA expression limited to the NRT of rats sacrificed 7 days after CACI. The described correlations between morphologic evidence of sprouting of GABAergic terminals, and clinical cessation of susceptibility to audiogenic seizures, as well as electrophysiologic demonstration of the return of $GABA_A$ inhibitory function in the hippocampus indicate the regenerative effort of the brain tissue subjected to ischemia and provide criteria for evaluating various therapeutic measures in future studies.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02889-02 SB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Spreading Depression in Cardiac Arrest Cerebral Ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	N. Kawahara, M.D.	Visiting Fellow	SB, NINDS
Others	I. Klatzo, M.D.	Senior Scientist	SB, NINDS

COOPERATING UNITS *(if any)*
L.P.Penix, M.D., Epilepsy Research Branch, NINDS

LAB/BRANCH
Stroke Branch

SECTION
Cerebrovascular Pathophysiology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.4	PROFESSIONAL: 1.0	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of the spreading depression (SD) was investigated in rats subjected to cardiac arrest cerebral ischemia (CACI). The SD was induced by application of KCl either on the exposed dura of the parietal cerebral cortex or by KCl perfusion through the hippocampus Three days later, the animals underwent the CACI. With regard to the hippocampus, unilateral perfusion with KCl regularly resulted in induction of SD in the ipsilateral hippocampus, associated with marked elevation of glutamate. No such effect was observed in rats in which KCl had been replaced with physiologic saline solution. Animals with hippocampal KCl perfusion, followed 3 days later by CACI, showed significant protection of CA1 pyramidal neurons on the side of the perfusion. No such effect was observed in Krebs-Ringer perfused rats. The protective effect of KCl on CA1 pyramidal neurons was evident also following the cortical appli-cation, although the effect was more bilateral. The SD induced 3 days before CACI resulted in a marked reduction in the susceptibility of rats to audiogenic seizures (AuSz) when tested 24 hr after cardiac arrest insult.

To elucidate the protective nature of the SD, the brain tissue was studied in rats at various time intervals following induction of the SD and in various relevant control conditions. Our studies indicated a striking stimulation of protein synthesis in the hemisphere ipsilateral to SD, which was demonstrable only in animals with SD induction 3 days earlier. Elevation of protein sysntheis was absent in rats sacrificed 1 or 7 days after SD induction and in all control groups of rats.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02821-05 SB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Dynamics of Postischemic Calcium Accumulation and Protein Synthesis in Brain Tissue

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

P.I.:	I. Klatzo, M.D.	Senior Scientist	SB, NINDS

COOPERATING UNITS (*if any*)

LAB/BRANCH
Stroke Branch

SECTION
Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.20	PROFESSIONAL: 0.10	OTHER: 0.10

CHECK APPROPRIATE BOX(ES)
[ ] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Dynamics of pathological changes in brain tissue following cardiac arrest induced ischemia related to the findings of double tracer autoradiography of ^{45}Ca and [^{3}H] leucine uptake as respective indicators of ischemic injury and metabolic disturbance. Abnormal calcium accumulation, determined by ^{45}Ca uptake, was related to injured but still living neurons and to reactive glial elements. ^{45}Ca autoradiography confirmed a high sensitivity to neuronal injury of the nucleus reticularis thalami (NRT), hippocampal CA1 pyramidal layer, inferior colliculus, ventral thalamic nucleus (VTN), caudate nucleus and parietal cortex. [^{3}H]leucine incorporation revealed that an initially widespread inhibition of protein synthesis was followed by its considerable recovery. Observations concerning the hippocampal CA1 sector and VTN suggested that a significant degree of protein synthesis, maintained at the late stage after postischemic recovery, was related to survival and regeneration of neurons and not to the presence of glial elements.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02912-01SB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Observations on Brain-Derived Neurotrophic Factor (BDNF) in Cerebral Ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Nobutaka Kawahara, M.D..	Visiting Fellow	SB, NINDS
Others	Igor Klatzo, M.D.	Senior Scientist	SB, NINDS

COOPERATING UNITS *(if any)*
Stanley Wiegand, Ph.D., Susan Croll, Ph.D., Regeneron Pharmaceuticals, Inc, Tarreytown, NY

LAB/BRANCH
Stroke Branch

SECTION
Section of Neurocytobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies have generally been focused on the nature of defensive and regenerative mechanisms which can be activated in response to ischemic injury, as well as on potential therapeutic manipulation of such defensive responses. In these studies we were able to demonstrate participation of brain-derived neurotrophic factor (BDNF) in the development of enhanced neuronal resistance to ischemic injury, demonstrable 3 days after induction of the spreading depression in the cortex. In therapeutic trials, BDNF was administered via the microcanullae inserted sterotaxically into the ventral thalamic region of the rat subjected to cardiac arrest cerebral ischemia (CACI). The delivery of the BDNF commenced 3 days before the CACI and lasted till the sacrifice of the animal at 7 days after cardiac arrest. Using specific monoclonal antibody for GABA demonstrated a striking proliferation and sprouting of GABAergic terminals in the ventral thalamic nucleus in the vicinity of the site of BDNF delivery. Also, there was a conspicuous preservation of GABAergic neurons of the nucleus reticularis thalami on the side of BDNF application.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02454-14 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Human Pituitary Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward Oldfield, M.D.	Chief, SNB, NINDS
Other:	Zvi Ram, M.D.	Visiting Scientist, SNB, NINDS

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NINDS
Diagnostic Radiology, CC

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, CNP

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.30	0.18	0.12

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We investigated venous sampling of the pituitary venous drainage to aid in the diagnosis and treatment of patients with Cushing's syndrome. Over 700 patients have now received bilateral simultaneous inferior petrosal sinus (IPS) sampling. The results indicate that (1) the procedure can be performed successfully in all patients with Cushing's syndrome (successful sampling has been performed in over 99% of the patients in whom it has been attempted); (2) the procedure distinguishes patients with ectopic ACTH secretion from those with pituitary adenomas with nearly 100% accuracy; (3) IPS sampling successfully determines in which side of the pituitary gland microadenomas reside in patients with Cushing's disease with 70% accuracy; and (4) unilateral inferior petrosal sinus sampling, which is commonly used clinically, is frequently misleading. Repeat transsphenoidal surgery is successful in eliminating the hypercortisolism of Cushing's disease in about 70% of patients. This therapy for patients with Cushing's disease after previous pituitary surgery had not previously been examined. Repeated sella exploration in the early postoperative period in patients who did not respond to the first operation was shown to be successful in most patients who received it. The subset of patients who are most likely to have success with early repeat surgery can be selected based on the findings during the first operation. MRI scanning with and without gadolinium-EDTA was used to evaluate patients with Cushing's disease preoperatively. This technique permitted identification of the adenoma in about 55% of those patients with surgically proven microadenomas. Proper timing of the MRI after administration of gadolinium-EDTA was critical in the optimal use of the technique. Pituitary adenomas were detected in 10% of 100 normal subjects with MRI scanning with contrast. The endocrine aberration in pituitary tumors and ectopic ACTH secreting tumors that cause Cushing's syndrome is loss of normal negative feedback regulation by cortisol. To investigate the basis of this, the structure of the proopiomelanocortin promoter region was investigated in pituitary and extra pituitary ACTH-producing tumors and demonstrated to be normal. Intraoperative ultrasound, using a prototype 12 Mhz probe developed for transsphenoidal surgery, was shown to provide a way of detecting and localizing very small tumors in the pituitary gland during surgery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02674-10 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monoclonal Antibody-Toxin Conjugates for Tumor Therapy *in vivo*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS
Others:	Dianne Newton, Ph.D.	Staff Fellow, SNB, NINDS
	Susanna Rybak, Ph.D.	Special Expert, SNB, NINDS
	Massimo Gadina, Ph.D.	Special Volunteer, SNB, NINDS
	You-Neng Wu, Ph.D.	Visiting Associate, SNB, NINDS

COOPERATING UNITS *(if any)*

Alfacell, Bloomfield, New Jersey

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.78	**PROFESSIONAL:** 2.38	**OTHER:** 0.40

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies selectively bind tumor cell differentiation antigens *in vitro* and *in vivo*. Since natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells we have devised methods of linking extremely toxic proteins to the antibodies to selectively kill tumor cells. We have succeeded in developing several new approaches to apply immunotoxins *in vivo*. (1) Cloning toxins, then altering their structure at the gene level to decrease non target cell toxicity; (2) intrathecal administration of immunotoxins for therapy of brain tumors that kill 2-5 logs of tumor cells in animal models; (3) preparation of genetically engineered immunotoxins for clinical trials of human brain tumor patients; (4) prevention of an immune response against immunotoxin with anti-CD4 antibodies; (5) specific deletion of Purkinje cells in rats, guinea pigs, and rhesus monkeys; (6) use of human cytotoxic proteins such as RNase linked to antibodies to selectively target cells; and (7) understanding the mechanism of human RNase neurotoxins.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02708-09 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Vascular Permeability Factor/Vascular Endothelial Growth Factor in the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marsha Merrill, Ph.D., Biologist	SNB, NINDS
Others:	John Heiss, M.D., Senior Staff Fellow	SNB, NINDS,
	Mima Bacic, M.D., Visiting Associate	SNB, NINDS
	Nancy Edwards, B.A.,Biologist	SNB, NINDS
	Efstathios Papavassiliou, M.D., Vis. Fell.	SNB, NINDS
	Edward H. Oldfield, M.D., Chief,	SNB, NINDS
	Seth Zeidman, M.D., Clinical Associate	SNB, NINDS

COOPERATING UNITS *(if any)*

Laboratory of Cardiovascular Science, NIA, NIH
Pulmonary Branch, NHLBI, NIH

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Tumor Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.15	4.0	1.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF), has been proposed to be a mediator of endothelial proliferation and angiogenesis in normal and diseased states, and to have a role in the development of tumor-associated vascular hyperpermeability. VEGF/VPF exists as multiple forms due to alternative splicing of the gene, and these forms exhibit different biologic behaviors. We have determined that the expression of these multiple forms is organ-specific, suggesting that the functions of VEGF/VPF are organ-specific as well. In tissue culture cells hypoxia ($<$12% oxygen) is a potent inducer of VEGF/VPF gene expression. This may be part of the mechanism involved in hypoxia-induced cerebral angiogenesis and in ischemia-induced hyperpermeability. A nonreplicating adenovirus which carries the VEGF/VPF gene is capable of infecting multiple cell types, expressing functional VEGF/VPF protein, and stimulating angiogenesis and permeability in bioassays. This construct provides a useful tool for studying the effects of VEGF/VPF in normal brain, as well as the possibility of gene therapy for cerebral ischemia. Antibody studies demonstrate that VEGF/VPF is responsible for $\sim$ 75% of the permeability inducing activity produced by brain tumor cells. Hyperpermeability associated with brain tumors is a significant cause of morbidity and mortality in this disease. Steroids are the standard treatment for this condition, but the mechanism of this steroid effect is poorly understood. We have determined using a brain tumor model that this inhibition of tumor capillary permeability by steroids is mediated through the glucocorticoid receptor, a finding that has important implications for the proper clinical management of brain tumor patients.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02739-08 SNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical and Laboratory Investigation of Central Nervous System Vascular Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward H. Oldfield, M.D.,	Chief, SNB, NINDS
Others:	Ryszard Pluta, M.D.,	Visiting Associate, SNB, NINDS
	Tom Manski, M.D.	National Naval Medical Ctr
	Robert Boock, Ph.D.	Staff Fellow, SNB, NINDS
	Kourosh B. Afshar, M.D.	Clin. Assoc., SNB, NINDS
	Marston Linehan, M.D.	Surgical Branch, NCI
	Berton Zbar, M.D.	Senior Investigator, NCI

COOPERATING UNITS *(if any)*
Diagnostic Radiology Department, CC, Experimental Therapeutics Branch, NINDS
Surgery Branch, National Cancer Institute; National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH
Surgical Neurology Branch, NINDS

SECTION
Clinical Neurosurgery Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.23	PROFESSIONAL: 2.13	OTHER: 0.10

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Endothelial-derived relaxation factor nitric oxide (NO) was shown to mediate autoregulation and chemoregulation of cerebral blood flow. NO synthase (NOS) immunoreactivity was demonstrated in the nerve plexus in the adventitia of the circle of Willis in primates. In a primate model of subarachnoid hemorrhage (SAH) adventitial disappeared on day 7 with the development vasospasm and did not return on day 14 with resolution of vasospasm, suggesting that NO loss plays a central role in the pathogenesis of cerebral vasospasm after SAH. Thus, direct replacement of NO should reverse the vasospastic effect of any NO loss. In the primate model of vasospasm, intra-arterial infusions of NO solution and NO donor solution reversed arteriographic cerebral vasospasm, significantly increased cerebral blood flow, and decreased cerebral blood flow velocity. These findings further support a central role of NO in the pathogenesis of cerebral vasospasm and suggest the potential of a regional NO therapy for cerebral vasospasm. We have explored the effects of the putative agents of vasospasm, oxyhemoglobin and its breakdown product methemoglobin in cell culture. These cultures studies are used to examine the possibility that vasospastic agents (e.g., endothelin) may be released from tissues exposed to oxyhemoglobin and methemoglobin. While exposure to hemoglobin does not directly increase endothelin levels, hypoxia, a condition associated with a decrease in cerebral blood flow, does cause dramatic increases in endothelin. This agent may be responsible for the secondary effects associated with vasospasm after SAH. A specific type of cranial dural arteriovenous fistulas was identified and shown to be treated effectively by simple interruption of the intrathecal venous drainage, a much simpler and safer procedure than the surgical procedure previously used to treat these patients.The lasting efficacy of a simple surgical procedure for patients with spinal dural arterovenous fistulas, interruption of the vein cleaning the fistula intradurally, was demonstrated. A new type of tumor associated with VHL syndrome, low grade adenomocarcinoma of the endolymphatic sac, was identified.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02781-06 SNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tissue Implantation in Parkinsonian Models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	Daniel Lieberman, M.D.	Staff Fellow, SNB, NINDS
	Alex Cummins, M.S.	Biologist, SNB
	Hideki Takubo, M.D.	Visiting Associate, NINDS

COOPERATING UNITS *(if any)*
David Jacobawitz, Clinical Neuropharmacology, NIMH, Charles Gerfen, Neurophysiology, NIMH, Ivan Mefford, Neurochemistry, NIMH

LAB/BRANCH
Surgical Neurology Branch, NINDS

SECTION
CNS Transplantation Unit

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.44	PROFESSIONAL: 1.41	OTHER: 1.03

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Brain grafting has emerged as a novel therapy for patients suffering from Parkinson's disease who are refractory to medical therapy. Behavioral recovery following caudate cavitation in Parkinsonian monkeys has focused our attention on potentially beneficial host responses to grafting. Following injury, the CNS produces neurotrophic factors which promote neurite outgrowth and glial proliferation. We have explored the therapeutic potential of growth factors in preventing or reversing biochemical and behavioral parameters in rodent models of Parkinson's disease. To further investigate the physiology of nerve growth factors, we have lesioned the dopamine system in mice with MPTP and measured the transcription of brain-derived neurotrophic factor (DBNF) and neurotrophin-3 using Northern blotting. To deliver growth factors to gray matter we are developing methods for convection-enhanced direct infusion and *ex vivo* gene therapy. We used convection to enhance the distribution of large molecules injected into the striatum in rats, measured using immunohistochemistry and quantitative autoradiography. We are beginning to explore the viability and biology of fetal human glial cells after transplant in mice, rats, and monkeys as an alternative paradigm to continuously deliver proteins to the degenerating dopamine system. Recent electrophysiologic and anatomic studies have shown hyperactivity of neurons in the subthalamic and globus palladium interna nuclei produce the symptoms of Parkinson's disease. Accordingly, we are exploring the use of excitatory amino acids to destroy the globus pallidus interna in monkeys as a novel therapy.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02812-05 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Pentobarbital Effects on Damage of the Primate Brain by Fractionated Whole Brain Radiation

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	Calvin Hawkins	Bio Lab Technician, SNB, NINDS

COOPERATING UNITS (*if any*)

Radiation Oncology Branch, NCI

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.53	0.08	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Radiation therapy remains the single most effective treatment for malignant brain tumors, but in many cases, toxicity to normal brain impedes therapeutic doses sufficient for local control to be achieved. A substantial effort has been directed toward overcoming the unfavorable side effects of brain tumor radiation therapy. Data from our institute and others indicate that the concomitant application of pentobarbital anesthesia during cerebral irradiation reduces the toxicity of the ionizing radiation. Although mechanisms of this phenomenon remain unclear, it seems to arise from general suppression of brain synaptic activity or metabolism. After baseline MRI scans of the brain and neuroendocrine testings, primates (*Macaca mulatta*) undergo whole brain X-irradiation in 10 daily fractions, 360 rads each, total dose of 3600 rads. The monkeys in the study group were anesthetized with pentobarbital during the irradiation whereas the animals in the control group received ketamine. Each group consists of 6 animals. Neuroendocrine testing and MRI scan follow-up studies are performed at 3, 6 12, 18 and 24 months after irradiation. Quantitative histology will be done on the capillary bed, glial and neuronal populations after sacrifice.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02813-05 SNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacokinetics of Direct Brain Infusion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	Douglas W. Laske, M.D.	Senior Staff Fellow, SNB, NINDS
	Dan Lieberman, M.D.	Staff Fellow, SNB, NINDS
	Orhan Ilercil, M.D.,	Clinical Associate, SNB,NINDS
	Aytac Akbasak, M.D.	Visiting Associate, NINDS
	Bob Boock, Ph.D.	Senior Staff Fellow, SNB, NINDS
	Paul Morrison, Ph.D., Robert Dedrick, Ph.D.	Biomedical Engineering, RR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Surgical Neurology Branch, NINDS

SECTION
Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.27	PROFESSIONAL: 1.24	OTHER: 0.03

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

For many compounds (neurotrophic factors, antibodies, growth factors, genetic vectors, enzymes) minimal diffusion in the brain severely limits drug distribution after direct drug administration in to brain parenchyma. We systemically investigated convection, molecular transport with bulk flow of fluid, to enhance the distribution of large and small molecules, indium^{111}-transferrin (In^{111}-Tf; MW 80,000) and C^{14}-sucrose (MW 359), by maintaining a pressure gradient during interstitial infusion to generate bulk flow through the brain interstitium. The volume of distribution (V_d) containing $\geq$ 1% of infusate concentration increased linearly with the infusion volume (V_i) for In^{111}-Tf ($V_d/V_i = 6.1$) and C^{14}-sucrose ($V_d/V_i = 14.1$). 24 hr after infusion, the distribution of In^{111}-Tf increased, became more homogeneous, and penetration into gray matter occurred. By using convection to supplement simple diffusion, greatly enhanced distribution of large and small molecules can be achieved in the brain while achieving drug exposure orders of magnitude greater than systemic exposure. Convection-enhances distribution was shown to be an effective technique to homogeneously deliver large and small molecules in the gray matter of rats and non-human primates.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02814-05 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Abnormalities in Primary Glial Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Iqbal U. Ali, Ph.D.	SNB, NINDS
Others:	Abha Saxena, Ph.D.	Visiting Associate, SNB,NINDS
	Joan Barrick, B.S.	Biologist, SNB, NINDS
	Cindy Piccirilli, M.D.	Resident, NNMC
	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
	William Stettler-Stevensen, M.D.	NCI
	James Robertson, M.D. ,	Chairman, Dept. of NS, University of Tennessee

COOPERATING UNITS *(if any)*

University of Tennessee, Memphis, Tennessee
LCMB, NCI, NIH, Bethesda, Maryland

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.58	1.08	0.50

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glioblastomas are extremely complex and malignant neoplasms. We have taken several approaches to understand and identify, at a molecular level, the underlying mechanisms that translate into the malignant behavior of these tumors.

1. Immunohistochemical analysis of the p53 protein showed a heterogeneous pattern of subcellular compartmentalization of glioblastomas. Tumors with one single wild type allele of p53 and deletion of chromosome 17 display predominantly cytoplasmic, nuclear, or both cytoplasmic and nuclear reactivity. Furthermore, tumors with mutations in the same codon of p53 display very different staining patterns. These data suggest that the microenvironment of a particular tumor is important in determining the subcellular localization of p53.
2. Twenty tumors were analyzed for collagenase IV and Timp-2 expression. Both these genes were generally overexpressed in glial tumors compared to the normal human. brain.
3. Eight matched pairs of primary and recurrent tumors were analyzed for allelic deletions on chromosomes 10 and 17. The data clearly demonstrated additional genetic abnormalities in recurrent tumors, which included amplification of the αPDGRF gene, point mutations of the p53 gene and overexpression of collagenase and Timp-2.
4. Analysis of metastatic brain tumors showed chromosome 17p deletions and/or p53 mutations in 60% of the tumors. Our data support the concept that p53 gene alterations may contribute to the metastatic spread in certain types of cancers.
5. A region of homozygosity on chromosome 10q was identified in primary glial tumors as well as in brain metastases suggesting the presence of a gene(s) on chromosome 10q that plays a role not only in glial tumorigenesis but also in homing of certain types of tumors to the brain.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02815-05 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Pituitary Conticotroph Adenomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Iqbal Ali, Ph.D.	SNB, NINDS
Others:	Joan Barrick, B.S.	Biologist, SNB, NINDS
	Abha Saxena, B.S.	Biologist, SNB, NINDS
	Edward Oldfield, M.D.	Chief, SNB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.58	PROFESSIONAL: 1.08	OTHER: 0.50

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cushing's disease is caused by the pituitary hypersecretion of ACTH and occurs predominantly in women. Patients are cured by surgical removal of an ACTH-producing adenoma, suggesting evolution and expansion of a genetically aberrant cell. However, hypothalamic dysfunction and excessive stimulation of anterior pituitary corticotrophs by one or more neurotransmitter substances may also lead to the development of corticotropic adenomas. Allelotyping of the pituitary tumors is being carried out by using restriction fragment length polymorphism (RFLP) analysis. Initial studies showed loss of heterozygosity of genes on chromosome 17 and point mutations in the p53 gene in two of the six Nelson's tumors.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02823-04 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antibody-Toxin Conjugates for the Treatment of Human Brain Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS
Others:	Doug Laske, M.D.	Senior Staff Fellow, SNB, NINDS
	Orhan Ilercil, M.D.	Clinical Associate, SNB, NINDS
	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
	David Katz, M.D.	Neuropathologist, OD, NINDS
	Cynthia Sung, Ph.D.	Staff Fellow, PEIB
	Robert Dedrick, Ph.D.	Senior Staff Fellow, PEIB

COOPERATING UNITS *(if any)*

Diagnostic Radiology; Nuclear Medicine Department; National Cancer Institute, Hafslund Nycomed

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.57	PROFESSIONAL: 1.27	OTHER: 0.30

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A phase I dose-escalation study of intrathecal therapy with the immunotoxin 454A12-RTA for leptomeningeal neoplasia has been completed. This compound is a conjugate of a monoclonal antibody against the human transferrin receptor and the recombinant ricin A chain protein toxin. Eight patients with leptomeningeal spread of systemic neoplasia were treated with a total of 10 different doses of intrathecal immunotoxin covering a 1,000-fold increase in drug dose (1.2 - 1,200 micrograms). No toxicity was detected until the highest doses were reached. Acute toxicity consisted of transient headache, vomiting and decreased mental status with elevated intracranial pressure which was responsive to steroids and cerebrospinal fluid (CSF) drainage. Bioassays of serial CSF samples from these patients against tumor cell lines *in vitro* revealed that patients CSF retained cytotoxic activity against tumor cells for approximately 48 hours after intraventricular administration of immunotoxin. In addition, *in vitro* testing of 454A12-RTA against tumor cells harvested from the CSF in 3 study patients revealed tumor cell sensitivity to the drug before and after treatment at concentrations of drug much lower than the concentration achieved in CSF. Four patients had decreased lumbar CSF tumor cell counts, the most dramatic (>95%) occurring at the highest dose given. These results indicate that immunotoxin can be safely administered intrathecally in humans, retain bioactivity in the CSF, are cytotoxic to tumor cells from patients, and can reduce tumor burden after only a single dose. A new clinical trial of a genetically engineered immunotoxin, Tfn-CRM107, discovered within the branch has begun for treatment of parenchymal brain tumors.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02850 - 03 SNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Therapy of Disorders of the Central Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	Zvi Ram, M.D.	Visiting Scientist, SNB, NINDS
	Stuart Walbridge	Biologist, SNB, NINDS
	Kenneth Culver, M.D.	Senior Clinical Investigator, MB, NCI
	R. Michael Blaese, M.D.	Chief, Cellular Immunology, MB, NCI
	John Viola, M.D.	Clin. Assoc., SNB, NINDS
	Eric Oshiro, M.D.	Clin. Assoc., SNB, NINDS

COOPERATING UNITS *(if any)*
National Cancer Institute, Bethesda, Maryland
Genetic Therapy, Gaithersburg, Maryland

LAB/BRANCH
Surgical Neurology Branch, NINDS

SECTION
Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.9	PROFESSIONAL: 2.0	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects   [x] (b) Human tissues   [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New approaches to transfer genetic material into tumor and normal central nervous system (CNS) tissues is being explored for various diseases of the CNS. The mechanisms involved in effecting antitumor activity using the suicide gene transfer approach are investigated. Targeting of tumoral vascular tissue, choroid plexus epithelium, and normal CNS structures is being pursued. New viral vectors, such as adenoviruses, are also being evaluated for therapeutic approaches in the CNS. Clinical studies to treat malignant brain tumors are underway and a clinical trial for treating patients with leptomeningeal neoplasia is pending.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02854 - 03 SNB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Establishing the Physiology of Syringomyelia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	John D. Heiss, M.D.	Senior Staff Fellow, SNB
	Nick Patronas, M.D.	CC, Radiology
	Thomas Shawker, M.D.	CC Radiology
	William Kammerer, M.D.	CC Anesthesiology
	Robert Dedrick, Ph.D.	RR, BEIP
	Alec Eidsath, Ph.D.	RR, BEIP

COOPERATING UNITS *(if any)*
Diagnostic Radiology Department, CC
Anesthesiology Department, CC, BEIP

LAB/BRANCH
Surgical Neurology Branch, NINDS

SECTION
Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.21	PROFESSIONAL: 0.78	OTHER: 0.43

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to establish the mechanism(s) of progression of communicating syringomyelia. Communicating syringomyelia usually accompanies abnormalities at the craniocervical junction. Measurement of intraventricular pressure, intrathecal pressure, and intrasyrinx pressure is providing data which elucidate the hydrodynamic mechanism(s) of progression of syringomyelia. Radiographic testing, including MRI flow studies and ultrasonography, is demonstrating how pathologic anatomy alters normal cerebrospinal fluid flow. The effect of posterior fossa craniectomy, upper cervical laminectomy, and duraplasty on cerebrospinal fluid flow and pressure, syrinx size, and neurological function is being evaluationed. Twelve patients have been treated. Only one patient had communication between the 4th ventricle and the syrinx. Despite obstruction of CSF pathways at the foramen magnum, phase and cine-MRI demonstrated pulsatile syrinx and cervical subarachnoid CSF flow. Ultrasonographic measurements demonstrated tonsillar descent, cord and syrinx constriction, and caudal syrinx fluid flow during systole. CSF pressure measurements showed that intracranial pulse pressure was transmitted well to the cervical subarachnoid space and syrinx but poorly to the lumbar thecal space. Because intracranial pressure is transmitted despite obstruction of the subarachnoid space at the foramen magnum, we conclude that the cerebellar tonsils and the brainstem act on a partially enclosed spinal subarachnoid space to generate cervical subarachnoid CSF pressure waves. These waves compress the spinal cord from without, not from within, as has previously been considered to occur, to propel the syrinx fluid downward with each heartbeat. Syrinx progression occurs as a consequence. Craniocervical decompression and duraplasty improved cerebrospinal fluid flow at the foramen magnum in all patients. All syrinxes decreased in size following surgery. All pressure measurements have been performed without complication, including post-operative measurement of cervical and lumbar pressure.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02855-03 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interstitial Therapy with Targeted Protein Toxins for Malignant Brain Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Douglas Laske, M.D.	Senior Staff Fellow, SNB, NINDS
Others:	Eward H. Oldfield, M.D.	Chief, SNB,NINDS
	Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS
	Orhan Ilercil, M.D.	Clinical Associate, SNB, NINDS
	David Katz, M.D.	Neuropathologist, OD, NINDS
	Nicholas Patrons, M.D.	Radiologist, CC

COOPERATING UNITS *(if any)*

Department of Radiology, CC

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.86	1.41	0.45

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are investigating a new approach for the treatment of brain tumors which utilizes new delivery approach for distribution of a class of potent, targeted anti-cancer compounds, called targeted protein toxins. Preclinical *in vitro* and *in vivo* experiments of toxins targeted to the transferrin receptor and epidermal growth factor (EGF) have demonstrated significant antitumor activity against a variety of tumor types including malignant gliomas. New methods of drug delivery have been developed to deliver these agents to brain tumors, and in vivo imaging method are being developed to demonstrate drug distribution in patients. We have initiated a dose escalation trial of regional therapy with the targeted protein toxin transferrin-CRM107 (Tf-CRM107) for the treatment of recurrent malignant brain tumors. Tf-CRM107 is a conjugate of human transferrin (Tf) and diphtheria toxin with a point mutation (CRM107). Tf-CRM107 binds to the transferrin receptor, which facilitates iron uptake and is present in higher number on tumor cells than on the normal cells of the brain, and the diphtheria toxin mutant kills these tumor cells to which the Tf-CRM107 binds. The purpose of the study is to evaluate the toxicity of Tf-CRM107 when delivered by intratumoral and peritumoral slow interstitial infusion in a dose escalation schedule and to assess antitumor activity in these patients. Twenty-one patients with malignant brain tumors refractory to standard therapy (surgery, radiation ± chemotherapy) have been treated. Results indicate that therapy with Tf-CRM107 effects tumor responses, without severe neurologic or systemic toxicity. A multicenter Phase II study is being planned. In addition, an EGF-target toxin is being prepared for clinical trial. Synergism between targeted protein toxins and other antitumor reagents including standard chemotherapy drugs and retinoids is under investigation.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02859-03SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Programmed Cell Death in the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard J.Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS
Others:	Bruno Dipasquale, M.D.	Visiting Associate, SNB, NINDS
	Katherine A. Wood, Ph.D.	Visiting Fellow, SNB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS: 1.9	PROFESSIONAL: 1.9	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied programmed cell death in the nervous system and the biochemical mechanism of apoptosis in general. To approach the nervous system, more sensitive and *in situ* methods are needed to identify cells undergoing programmed cell death. We have developed two new methods to identify apoptotic cells under the microscope. (1) We have found that thymocyte-programmed cell death can be followed morphologically with Nomarski optics and that the thymocyte death resembles neuronal cell death. The morphologic analysis of nuclear disintegration has allowed us to test whether cell death is due to production of a toxic factor or due to the loss of a protective factor. Using the new microscopic method to identify apoptosis, the nuclei in the heterokaryons were found to follow the original and distinct fate of the parent cells and not to transfer apoptosis nor viability between nuclei. This new method also allowed us to identify apoptosis as the method of cerebellar granule cell death after MPP^+ treatment *in vitro*. (2) We have also developed a molecular detection method to measure DNA strand breaks *in situ*. This allows us to examine brains of animals undergoing neurodegenerative changes during ischemia, MPTP treatment, and during development. This new method should illuminate the role apoptosis plays during development and during various disease states of the nervous system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02868-03 SNB

PERIOD COVERED
October 1, 1993 Through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Semi-Chronic Intracortical Electrical Stimulation of the Visual Cortex of a Blind Volunteer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Conrad Kufta, M.D.	Medical Officer, SNB, NINDS
Others:	Daniel O'Rourke, M.D.	University of Pittsburgh, PA
	Martin Bak	Electrical Engineer, LNLC, NINDS
	Edward Schmidt, Ph.D.	Biological Engineer, LNLC, NINDS
	F. Terry Hambrecht, M.D.	Head, Neuroprothesis, NINDS

COOPERATING UNITS *(if any)*
University of Pittsburgh

LAB/BRANCH
Surgical Neurology Branch, NINDS

SECTION
Clinical Neurosurgery Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS: 0.18	PROFESSIONAL: 0.18	OTHER: 0

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to evaluate the feasibility of a visual prosthesis for totally blind individuals by stimulating chronically implanted microelectrodes in the visual cortex. A 42-year-old woman who has been blind for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensation of light called phosphenes. Phosphenes were produced with 34 of the 38 electrodes with currents that were 100 to 1000 times lower than had been reported for surface stimulation of the visual cortex. Additional blind patients need to be tested before we will know if intracortical microstimulation (ICMS) of the visual cortex is a feasible technique for producing a visual prosthesis. However, all the tests performed to date indicate that ICMS may be feasible.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02880-02 SNB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inducers of Differentiation of Malignant Brain Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Zvi Ram, M.D.	Visiting Scientist, SNB, NINDS
Others:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
	Stuart Walbridge, B.S.	Biologist, SNB, NINDS
	John Viola, M.D.	Clinical Associate, SNB, NINDS
	Eric Oshiro, M.D.	Clinical Associate, SNB, NINDS
	Dvorit Samid, M.D.	Clinical Pharmacology Branch, NCI
	Charles Myers, M.D.	Clinical Pharmacoogy Branch, NCI

COOPERATING UNITS *(if any)*

National Cancer Institute

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.55	PROFESSIONAL: 1.50	OTHER: 0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phenylacetate is a natural compound which has a profound effect on tumor growth. In collaboration with Drs. Samid and Myers of the NCI, we evaluated the *in vivo* effect of phenylacetate on prevention of tumor growth and antitumor efficacy against established tumors in an animal model.

Phenylacetate induced differentiation of the malignant tumor cells and extended survival when given simultaneously with tumor inoculation or as treatment to established tumors. No toxicity was associated with the therapy and therapeutic drug levels were achieved in the plasma and CSF. Electron microscopy of treated tumors showed striking hyperplasia of the rough endoplasmic reticulum as an *in vivo* marker of tumor differentiation. *In vivo* proliferation assays demonstrated a significant decrease in the mitotic index of treated tumors.

(This project was completed as of January 1994.)